Medical Library SEP 25 1944

VOLUME XX, No. 5 WHOLE No. 120

SEPTEMBER, 1944

THE AMERICAN JOURNAL OF PATHOLOGY

Official Publication of
The American Association of Pathologists and Bacteriologists

BOARD OF EDITORS

CARL V. WELLER, Editor-in-Chief
MALCOLM H. SOULE, Assistant Editor

J. HAROLD AUSTIN
PAUL R. CANNON
HOWARD T. KARSNER
TRACY B. MALLORY
SHIELDS WARREN
HARRY M. ZIMMERMAN

Editorial and Publication Office
EAST UNIVERSITY AVENUE, ANN ARBOR, MICHIGAN
Issued Bimonthly Annual Subscription in U. S. A. $8.00

Entered as second class matter, January 23, 1941, at the Post Office at Ann Arbor, Michigan, under the Act of March 3, 1879

COPYRIGHT, 1944
BY THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

This is the Microscope You are Waiting for

These are the lenses that make up the heart of a microscope. Assembled and mounted on the stand they become a B&L Microscope . . . the microscope you have been planning to purchase when restrictions are lifted.

It will represent the best and the latest in the application of optical science to microscopy. From the initial mathematical calculations to the final inspection each B&L Microscope will always measure up to this standard and each is the product of the experience that has gone before.

There are the facilities of America's first and finest optical glass plant, new methods of manufacture such as the diamond milling of opt parts, new materials such as the B&L low t perature lens cement and other new techniq All these things, and more, will be combined give you the finest optical equipment that can built.

BAUSCH & LOM

OPTICAL CO., ROCHESTER, N.Y.

Makers of Optical Glass and a Complete Line of Optical Instruments for Military Use, Education, Research, Industry and Eyesight Correction and

ng of opa
&L low t
w techniq
combined
t that cas

THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XX SEPTEMBER, 1944 NUMBER 5

THE DEVELOPMENT OF HEPATIC CIRRHOSIS IN DOGS AFTER HYPOPHYSECTOMY

ITS ASSOCIATION WITH UNANTICIPATED, COINCIDENTAL HYPOTHALAMIC INJURY *

IRVING GRAEF, M.D., JUAN NEGRIN, JR., M.D.,† and IRVINE H. PAGE, M.D.‡
(From the Department of Pathology, New York University College of Medicine, New York, N.Y.)

During the course of studies on the influence of hypophysectomy on experimental renal hypertension in dogs, post-mortem examination revealed fatty cirrhosis of the liver in 3 animals. Thereafter another animal with early cirrhosis was encountered in Dr. Maurice Bodo's colony of hypophysectomized dogs and all four instances were reported briefly in 1939.[1] Since then another hypophysectomized dog with cirrhosis was encountered by Dr. Allen Keller at the University of Alabama, and he has kindly permitted us to include his case in this report.

When presumably comparable dogs were examined and no hepatic changes were observed, and it appeared unlikely that reduction or ablation of the hypophysis was related to the hepatic changes, further study was undertaken to find some other possible basis for the hepatic changes. Fifteen additional hypophysectomized dogs were available through those provided by Drs. Bodo and Shannon and one of us (I. H. P.). Hepatic fibrosis occurred in one instance that will be described later. However, few of the additional dogs were kept alive beyond 6 months and only 3 beyond 1 year. In this paper we are reporting the detailed findings in the 5 cirrhotic and, in addition, 6 noncirrhotic dogs, including among the latter 2 surviving 3½ to 5¼ years.

Special attention was directed to the diencephalon because of its important rôle in many vegetative functions and in certain aspects of metabolism. The possibility of a neuropathologic basis for hepatic changes gains further support from the occurrence of Wilson's disease in man (lenticular degeneration and hepatic cirrhosis) as well as the occurrence of fatty livers in certain neuropathologic states.[2, 3]

* Received for publication, November 1, 1943.
† Dazian Fellow in Pathology, New York University College of Medicine, 1939–41.
‡ Lilly Clinic, Indianapolis City Hospital, Indianapolis, Indiana.

Material and Methods of Study

The animals were chiefly adult mongrel dogs from the city of New York, maintained on an abundant diet of hospital scraps (meat scraps and bones, bread, vegetables); to some, Purina dog biscuits were given daily and meat three times weekly. Hypophysectomy was performed on most of the animals by Dr. Joshua E. Sweet, using a subtemporal approach.[4] This consists of the removal of the zygomatic arch by subperiosteal resection of the coronoid process close to the base. The skull is then trephined and the dura removed. The extirpation of the pituitary body is accomplished with the aid of a double spoon or curette. Rotation of the spoon after its edge is inserted underneath the pituitary capsule removes the gland and transects the infundibular stalk. Other surgical and experimental procedures related to the production of hypertension are noted in Table I-A.

The dogs furnished by Drs. Keller and Shannon were mongrels from Birmingham, Alabama, and had been subjected to transection of the pituitary stalk with hypophysectomy by the nasopharyngeal route.

In addition to the brains of the 11 animals that had been operated upon, those of 4 additional dogs were examined. Two were from animals dying during unrelated experiments, and 2 were from animals that died during renal hypertension. One of the latter dogs appeared to be in uremia at the time of death. All served as controls in standardizing the neuropathologic study, and the hypertensive animals also served as controls for the influence of hypertension. There were no pathologic changes in the pituitary gland or the hypothalamus.

The brains were fixed in 10 per cent Formalin (4 per cent solution of formaldehyde), after macroscopic examination. The tissue chosen for study was a block including the hypothalamic region; it was limited anteriorly by the rostral part of the optic chiasma, posteriorly by the caudal portion of the mammillary bodies. Laterally it extended to the level of the temporal poles and superiorly to the floor of the lateral ventricles, or to the outer surface of the cortex in 3 dogs. Serial celloidin, paraffin, or frozen sections were cut. For study of nerve cells and Nissl bodies the Nissl method was used. Myelinated nerves were stained with Loyez'[4a] or Weil's method. As general stains, hematoxylin and eosin, Masson's trichrome stain, and Mallory's phosphotungstic acid hematoxylin were used. Prussian blue and mucicarmine stains were employed occasionally for demonstration of iron or mucin. Frozen sections of the liver were stained for fat with Sudan IV, and paraffin sections were stained by a variety of connective tissue stains. The sella turcica and covering dura mater of 7 dogs were examined in serial sections for pituitary remnants after decalcification. In the other 4

dogs only the remainder of the infundibular stalk was available for such study; the sella turcica had been inspected but not saved.

Results

Tables I-A and I-B present a summary of the clinical and pathologic findings in the 5 animals with cirrhosis examined from 4½ months to 3 years after hypophysectomy. Tables II-A and II-B are based on 6 animals without cirrhosis examined from 10 months to 5¼ years after the operation. Since the findings reported here were not anticipated, there are many deficiencies in our clinical data and some in our post-mortem material. In a few animals, however, special attention was paid to symptoms and changes reflecting hypophyseal or hypothalamic injury, and the available notes are recorded in each table.

Generalized obesity was observed during the post-operative course of 8 dogs. It did not appear in 2 dogs, surviving only 10 days and 1 month respectively, or in 1 dog surviving 6 months. The fur of the 9 animals surviving 4½ months or longer became soft and luxuriant. No changes were observed in the coats of the 2 animals surviving 10 days and 1 month respectively. Diabetes insipidus was of variable onset, degree and duration in 8 dogs, all surviving 4½ months or longer. It was absent in 1 dog surviving hypophysectomy for 8 months and did not appear in the dog surviving 10 days, or in the dog surviving 1 month. These and the visceral findings are analyzed separately in the following sections. Correlations were made with the extent of the hypophysectomy and any coincidental hypothalamic lesions.

Liver

The lesions in the dogs with cirrhosis (Table I-A) varied considerably (Figs. 1 to 6). The mildest change was found in dog 4, sacrificed 11 months after operation. There was widespread fatty infiltration of the liver and on section increased lobular marking, suggestive of early cirrhosis, was visible (Fig. 3). Histologic study revealed a general increase of portal and periportal connective tissue with strands of young cellular connective tissue surrounding the hepatic lobules (Figs. 4 and 5). The latter appeared somewhat smaller than in comparable normal animals.

The remaining four livers were reduced in size and had macroscopic nodular cirrhosis, and protruding nodules of yellow tissue were visible through Glisson's capsule. All four also showed marked fatty deposit (neutral fat) in the parenchyma. The deposits of connective tissue were irregular (Figs. 2 and 6), being both monolobular and multilobular, involving the portal areas preponderantly in some portions, and irregularly traversing the parenchyma in others. No signs of recent

TABLE I-A

Clinical-Pathologic Observations on Cirrhotic Dogs

Experimental procedures			Clinical notes					
Dog no. and sex	Survival time after hypophysectomy	Renal and other	Changes in weight	Diabetes insipidus	Estrus	Miscellaneous	Hypertension	Adr
1 F	3 yrs., 7 mos. Operated upon, 1/36; sacrificed, 8/39	Rt. artery clamp, 11/35 Lt. artery clamp, 2/36 Bilat. oophorectomy, 1/37 Rt. nephrectomy, 6/38 Liver taken for biopsy, 8/39	Initial wt., 11 kg.; no wt. gain until spaying, then rose to 12.8 kg.	Polyuria for a few weeks after hypophysectomy	Ceased	Insulin reaction not tested; no convulsion observed	Transitory after June, 1938	Mild atroph moder depleti phy af ticular
2 M	2½ yrs. Operated upon, 10/35; died, 4/38	Rt. artery clamp, 9/35 Lt. artery clamp, 10/35	Initial wt., 13 kg.; postoperative gain up to 17 kg.	Permanent		Insulin reaction not tested; no convulsion observed	Present before hypophysectomy; transitory for 3 mos. after, and ceased	Marked atroph glomer and fe fascicu preser
3 F	2⅛ yrs. Operated upon, 11/35; died, 3/38	Rt. artery clamp, 9/35 Lt. artery clamp, 12/35	Initial wt., 9.5 kg.; max. wt., 14.6 kg.; wt. at death, 13.9 kg.	Permanent, 0.4 to 0.6 liters output daily	Ceased	Convulsions on several occasions, unrelieved by intravenous glucose	Present before hypophysectomy; fell after to range of 140–160 mm. Hg	Marked atroph glomer and fe fascicu preser
4* F	11 mos. Operated upon, 11/37; sacrificed, 10/38	None	Initial wt., 8 kg.; increased and remained at 9.75 kg.	Temporary and severe for 2 mos.	Ceased	Increased sensitivity to insulin	No data	Marked atroph glomer and fe fascicu preser
5† F	4½ mos. Operated upon, 5/39; died, 10/39	None	Initial wt., 10 kg.; gain to 13.6 kg. at death	Delayed onset of mild and permanent type	Ceased	Insulin reaction not tested	No data	Contra micr study

*From Dr. Bodo
†From Dr. Keller

degenerative or inflammatory alterations were found in four. In dog 4 a single microscopic focus of suppuration was encountered in one of five blocks. No bacteria were demonstrable in paraffin sections stained with Giemsa's stain.

The larger and smaller bile ducts and portal veins were unaltered. The smaller radicles were caught in the proliferated connective tissue. In some places remnants of lobules were discerned but their constituent

TABLE I-A

Clinical-Pathologic Observations on Cirrhotic Dogs

Significant post-mortem visceral findings				
Adrenal	Gonads	Liver	Thymus	Spleen
Mild cortical atrophy, with moderate lipid depletion; atrophy affects reticular zone	Not examined (removed 1 yr. after hypophysectomy and 2½ yrs. before death)	Unevenly distributed, sometimes multilobular, monolobular cirrhosis, chiefly portal; accompanied by reduction in size of the liver; irregular contraction of liver lobules and condensation of portal canals; some proliferation of bile ductules; marked increase of collagenous fibers; no inflammatory or necrotizing changes; moderate deposits of neutral fat globules in all lobules	Not examined	Marked atrophy of malpighian bodies and pulp cells
Marked cortical atrophy; only glomerular zone and few cells of fascicular zone preserved	Resting testis; no spermatogenesis beyond formation of spermatocytes	Widespread, monolobular and multilobular cirrhosis accompanied by marked deposits of neutral fat in liver cells; no inflammatory or necrotizing changes; considerable iron-containing pigment stored in macrophages in the proliferated connective tissue	Hyperplastic	Marked atrophy of malpighian bodies and pulp cells
Marked cortical atrophy; only glomerular zone and few cells of fascicular zone preserved	Prepubertal appearance of ovaries with many primordial follicles showing partial maturation	Diffuse monolobular and multilobular cirrhosis; fatty change marked in parenchyma; condensation of portal canals apparent in some areas; bile duct proliferation not definite; no inflammatory changes; mild focal bile and iron pigment storage	Persistent, uninvoluted	Marked atrophy of malpighian bodies and pulp cells
Marked cortical atrophy; only glomerular zone and few cells of fascicular zone preserved	Marked replacement fibrosis of ovary	Early monolobular cirrhosis; marked fatty deposit in trabeculae; periportal fibrosis moderate with little extension around lobules; one focus of "sterile" suppuration; focal bile duct proliferation	Hyperplastic	No atrophy
Contracted; no microscopic study	Ovary normal macroscopically; no microscopic study recorded	Macroscopically yellow and with prominent lobular markings, monolobular and multilobular cirrhosis; marked fat deposition in persistent parenchyma; no definite bile duct proliferation; no recent degenerative or inflammatory changes; no pigment storage	No observations	No observations

trabeculae appeared narrow, short and atrophic in spite of considerable amounts of fat within the parenchymal cells. Masses of greenish brown pigment were found as plugs in some bile canaliculi but this was an inconstant finding limited to dogs 1 and 3. Iron-containing pigment, stored in macrophages, was found in the portal areas or in Kupffer cells in 2 of the other dogs with cirrhosis. Similar pigment storage is not uncommon in "normal" street dogs in our experience. Proliferation

of the smaller bile ducts was also inconstant; it was seen as part of the cirrhotic process in 3 and it was doubtful or absent in the others.

In the proliferated connective tissue other types of cells were rare. Macrophages with iron-containing pigment, histiocytes and, very rarely, lymphocytes were sometimes found.

No special manifestations of hepatic dysfunction were observed in any of the animals. Neither ascites nor icterus had been observed.

There was no correlation between the occurrence of cirrhosis and the completeness of the hypophysectomy; in 2 dogs (3 and 4) no pituitary remnants were found, while in the other 3, microscopic remains were present varying from clusters of anterior pituitary cells in the adjacent dura to most of the pars tuberalis. It was estimated that 90 per cent or more of the pars glandularis was removed in each instance.

Length of survival after complete or incomplete hypophysectomy bore no special relation to the extent of the hepatic lesions, for fatty cirrhosis that was macroscopically visible was found after 4½ months in 1 animal (dog 5), and a less advanced hepatic lesion was found in

TABLE I-B

Neuropathologic Status in Cirrhotic Dogs

Dog no. and sex	Time after hypophysectomy	Post-mortem state of hypophysis	Hypothalamus
1 F	3 yrs., 7 mos.	Considerable acinar pituitary tissue of the pars tuberalis remains attached to the infundibulum	Besides diminution of cells in supra-optic nuclei, there is an erosion of the hypothalamus, extending up to the fornix on the right side. The n. ventro-medialis, periventricularis arcuatus, posterior portion of anterior hypothalamic area, medial and inferior portions of lateral hypothalamic area, are included. Defect crosses the midline and includes left n. ventro-medialis and periventricularis arcuatus. It diminishes in width and depth anteriorly towards optic chiasma and posteriorly towards mammillary bodies. On the left, the erosion leaves medial eminence intact.
2 M	2 yrs., 6 mos.	Clusters of chromophobic cells can be seen in some sections through the infundibulum	There is reduction in number of cells of supra-optic nuclei and an erosion is present in the inferior part of the hypothalamus, affecting the medial and inferior portions of right n. ventro-medialis and left n. periventricularis arcuatus, and n. ventro-medialis. At some levels the eminentia medialis and inferior part of wall of 3rd ventricle are eroded. Erosion consists of small cavities containing calcific deposits. Defect is covered by connective tissue continuous with dura of sella turcica.

another (dog 4) after 11 months. However, the 3 animals surviving 2½ years or more had nodular cirrhosis.

In the noncirrhotic group (Table II-A) the livers had minimal alterations; in 4 dogs the liver lobules seemed smaller because of closer approximation of the portal areas, suggestive of beginning condensation fibrosis, but no increase of connective tissue was found. The hepatic trabeculae appeared narrower. In 1 animal (3½ years after hypophysectomy) mild proliferation of bile ducts was found in the absence of any other change. Except for focal deposits in one liver, fatty deposition was notably absent.

Similar lack of correlation between completeness of hypophysectomy and hepatic status was noted in the noncirrhotic dogs. The liver was apparently normal after complete hypophysectomy in 1 animal (dog 6) surviving 5¼ years.

Another dog with only tiny microscopic clusters of residual pituitary tissue, surviving 3¾ years, developed minimal change in the liver consisting of a slight increase of periportal connective tissue without

TABLE I-B

Neuropathologic Status in Cirrhotic Dogs

Schematic drawings* to show the approximate location of the lesions in the hypothalamus from coronal (dogs 1, 2, 4) and sagittal sections (dogs 3, 5). Coronal drawings are shown with right side on the reader's left. Inclined lines on the drawings of sagittal sections represent levels at which coronal sections were made in the other dogs.

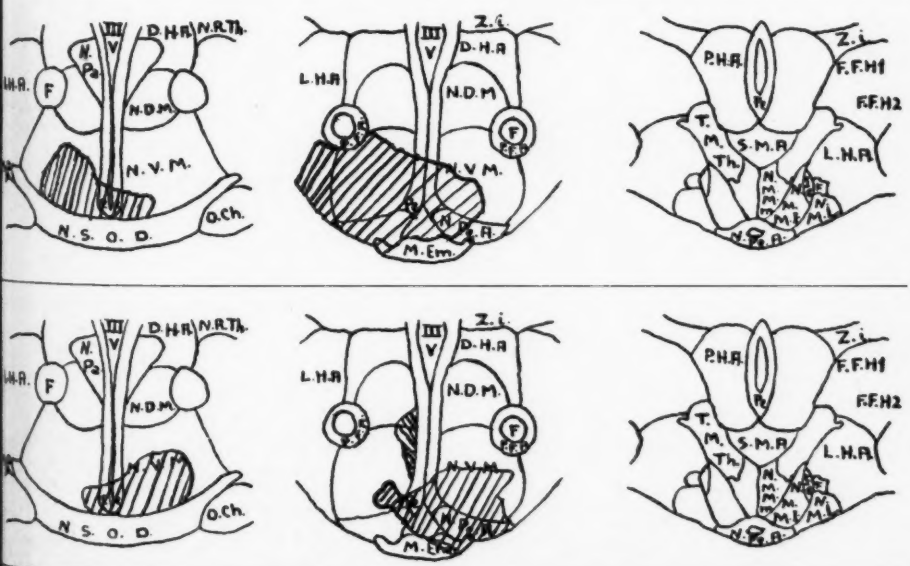


TABLE I-B (continued)

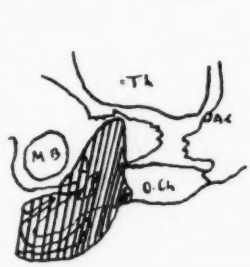
Dog no. and sex	Time after hypophysectomy	Post-mortem state of hypophysis	Hypothalamus
3 F	2 yrs., 4 mos.	No pituitary tissue found	An erosion is present between the optic chiasma and mammillary bodies. It includes the n. periventricularis arcuatus, ventromedialis, and medial portions of the lateral hypothalamic area. There is diminution of cells in the supra-optic nuclei.
4 F	11 mos.	No pituitary tissue found; operative specimen includes pars glandularis	A defect is found involving the inferior part of the floor of the 3rd ventricle and the infundibulum on both sides. The n. periventricularis arcuatus, both n. ventro-medialis and the most medial part of the lateral hypothalamic area on the right are included. There is reduction of cells in the supra-optic nuclei.
5 F	4½ mos.	Nests of chromophilic pituitary cells (eosinophils) and chromophobes in the dura covering the pituitary fossa, reported by Dr. Keller	The hypothalamus shows an erosion which extends to the supra-optic nuclei and the nuclei ventro-medialis, the dorso-medialis, and the periventricular arcuatus on both sides.

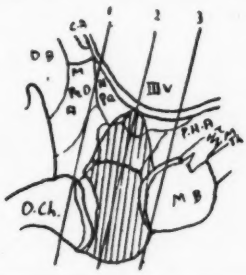
* Nomenclature is that given in "The Hypothalamus," published by the Association for Research in Nervous and Mental Diseases, Williams & Wilkins Co., Baltimore, 1940.

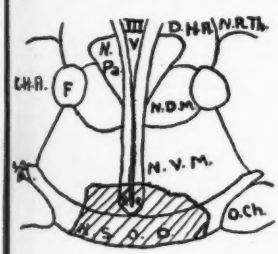
L.H.A.—Lateral hypothalamic area
F—Fornix
N.D.M.—Nucleus dorso-medialis
N.V.M.—Nucleus ventro-medialis
S.O.N.—Supra-optic nucleus
N.S.O.D.—Nucleus supra-opticus diffusus
O.Ch.—Optic chiasma
N.Pa.—Nucleus paraventricularis
Pe.—Periventricular system
P.F.A.—Perifornical area
D.H.A.—Dorsal hypothalamic area
N.R.Th.—Nucleus reticularis thalami
III V.—Third ventricle
Z.I.—Zona incerta
M.Em.—Median eminence
N.Pe.A.—Nucleus periventricularis arcuatus
PH.A.—Posterior hypothalamic area
F.F.H1, H2—Field of Forel H1, H2

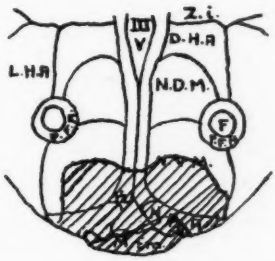
TABLE I-B (continued)

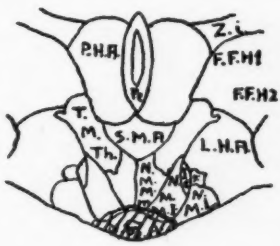
Schematic drawings* to show the approximate location of the lesions in the hypothalamus from coronal (dogs 1, 2, 4) and sagittal sections (dogs 3, 5). Coronal drawings are shown with right side on the reader's left. Inclined lines on the drawings of sagittal sections represent levels at which coronal sections were made in the other dogs.

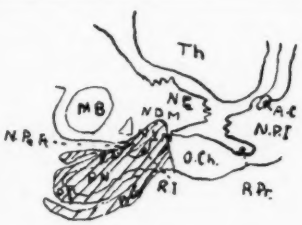


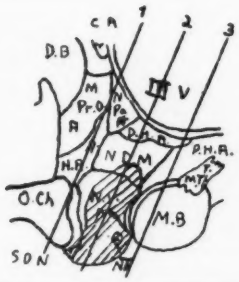












T.M.Th.—Mammillo-thalamic tract
S.M.A—Supramammillary area
N.M.Mm.—Nucleus mammillaris medialis (pars medialis)
N.M.Ml.—Nucleus mammillaris medialis (pars lateralis)
N.M.L.—Nucleus mammillaris lateralis
I.C.—Nucleus intercalatus
M.B.—Mammillary bodies
R.Pr.—Recessus preopticus
A.C.—Commissura anterioris
Th.—Optic thalamus
R.I.—Recessus infundibularis
P.N.—Pars nervosa
P.I.—Pars intermedia
P.G.—Pars glandularis
P.T.—Pars tuberalis
M.Pr.O.A.—Medial preoptic area
N.P.I.—Nucleus preopticus internus
D.B.—Diagonal band

TABLE II-A

Clinical-Pathologic Observations on Noncirrhotic Dogs

Experimental procedures			Clinical notes				
Dog no. and sex	Survival time after hypophysectomy	Renal and other	Changes in weight	Diabetes insipidus	Estrus	Miscellaneous	Hypertension
6 F	5¼ yrs. Operated upon, 1/36; sacrificed, 3/41	Rt. artery clamp, 3/35 Nephrectomy, lt., and adrenalectomy, lt., 4/35	Initial wt., (?); animal became obese and continued to gain before sacrifice	Mild, lasting 3 yrs.	Ceased	Insulin reaction not tested; spontaneous convulsion occurred once in 10/39	Modest, and maintained through 1939
7 F	3¾ yrs. Operated upon, 4/37; sacrificed, 1/41	Rt. artery clamp, 10/36 Lt. artery clamp, 10/36 Rt. artery clamp removed, 1/37 Lt. artery clamp removed, 2/37 Liver taken for biopsy, 8/39	Initial wt., (?); animal became obese and indolent	Moderate for 3½ yrs.	Ceased	Insulin reaction not tested; spontaneous convulsion occurred once in 1/40	From 2/37 to 4/37
8 M	8 mos. Operated upon, 6/39; died, 2/40. Cause: malignant hypertension	Rt. nephrectomy, 12/39 Lt. kidney wrapped in cellophane, 1/40	Initial wt., 10 kg.; 6 and 8 mos. after hypophysectomy, 11.5 and 12.8 kg. respectively	Absent (?)		Insulin reaction not tested; no convulsions observed; necrotizing arteriolitis in G.I. tract	Appeared 25 days before and was sustained
9 F	6 mos. 8 days Operated upon, 6/39; died, 12/39. Cause: malignant hypertension	Rt. kidney wrapped in cellophane, 8/39 Lt. kidney wrapped in silk, 9/39	None observed	Permanent	Not observed	Insulin reaction not tested; retinal detachment; renal insufficiency	Moderate, observed from 9/39 to 11/39
10 M	1 mo., 5 days Operated upon, 6/39; died, 7/39 Cause (?)	Lt. kidney wrapped in cellophane, 4/39	None	Not observed; no measure of intake and output of fluids		Insulin reaction not tested; no convulsions observed	4/28 to 6/15/39
11 M	10 days Operated upon, 9/30; died, 10/39. Cause: meningoencephalitis (?)	Kidneys denervated and wrapped with cellophane: rt., 10/38; lt., 1/39 Rt. nephrectomy, 4/39 Lt. kidney explanted, 5/39	None	Did not develop		Insulin reaction not tested; spontaneous convulsion occurred once in 10/39	Present from 1/19 to 4/18/39

TABLE II-A

Clinical-Pathologic Observations on Noncirrhotic Dogs

	Significant post-mortem visceral findings			
Adrenals	Gonads	Liver	Thymus	Spleen
Lt. removed Rt. normal	(?)	No visible fat; apparently normal	Persistent, uninvoluted	No atrophy No fibrosis
Marked cortical atrophy	Fibrous replacement of ovaries	Very mild proliferation of bile ducts and connective tissue, otherwise normal; no visible fat	Persistent, uninvoluted	No atrophy No fibrosis
Grosslyatrophic; not examined microscopically	Resting testis; spermatogonia show active division but spermatids absent	Mild lobular atrophy with moderate trabecular atrophy; widening of liver sinusoids and condensation of portal canals; fatty infiltration found in small zones in an occasional lobule	Uninvoluted, (hyperplastic ?)	No atrophy No fibrosis
Mild cortical atrophy affecting juxtamedullary zone	Active maturation of follicles; corpus luteum present; many primordial follicles visible	Irregular zonal atrophy; widespread focal periportal cellular infiltration; no definite fibrosis; no fatty infiltration	Not examined	No atrophy, active hemopoiesis, no fibrosis or chronic passive congestion
Grossly normal	Resting testis; interstitial cells scanty	Lobules somewhat reduced in size and liver cords are short and narrower than normal; no fatty infiltration; no increased fibrosis	Not found	No atrophy, no fibrosis or chronic passive congestion
Grossly normal; no sections available	Spermatocytes active in division; no spermatids in many tubules	Widespread narrowing of liver trabeculae but early stages of post-mortem autolysis are found in some lobules; no inflammatory changes; no fatty infiltration	Not found	No pigment storage or atrophy

TABLE II-B

Neuropathologic Status in Noncirrhotic Dogs

Dog no. and sex	Time after hypophysectomy	Post-mortem state of hypophysis	Hypothalamus
6 F	5¼ yrs.	Nests of chromophilic cells (eosinophils) and chromophobic cells were found in the dura beneath the tuber cinereum	No pathologic findings encountered in serial coronal sections except diminution of cells in supra-optic nuclei.
7 F	3¾ yrs.	Remnants of anterior pituitary cells (chromophilic alpha-cells and chromophobes are attached to the infundibulum)	There is diminution of cells in supra-optic nuclei. The most distal portion of the eminentia medialis, attached to the infundibulum, is separated from the rest of the brain. Lateral to this defect there is considerable increase in meningeal connective tissue, particularly rich in mononuclear cells with eosinophilic granules.
8 M	8 mos.	Attached to the infundibulum there are remnants of pituitary tissue consisting of chromophobes and eosinophilic granular cells	A defect of nerve substance affects the floor of the 3rd ventricle, from the level of the midline of the mammillary bodies to the anterior aspect of the infundibulum; it includes the wall of the 3rd ventricle and extends, on the right at some levels, to the medial part of the lateral hypothalamic area, through the n. ventromedialis and dorso-medialis and periventricularis arcuatus. Definite reduction of cells of supra-optic nuclei has occurred.
9 F	6 mos., 8 days	There are remnants of pituitary tissue attached to the infundibulum, represented by chromophilic (eosinophils) and chromophobic cells	A small erosion was found completely destroying the eminentia medialis. It extends to and opens the cavity of the third ventricle in the midline. There is apparent diminution of cells in the supra-optic nucleus.

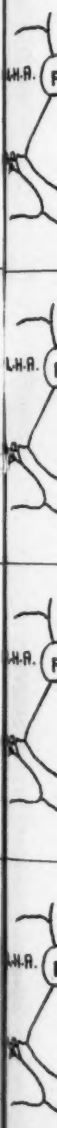


TABLE II-B

Neuropathologic Status in Noncirrhotic Dogs

Schematic drawings* to show the approximate location of the lesions in the hypothalamus from coronal sections through the diencephalon. Drawings are made with right side depicted on reader's left.

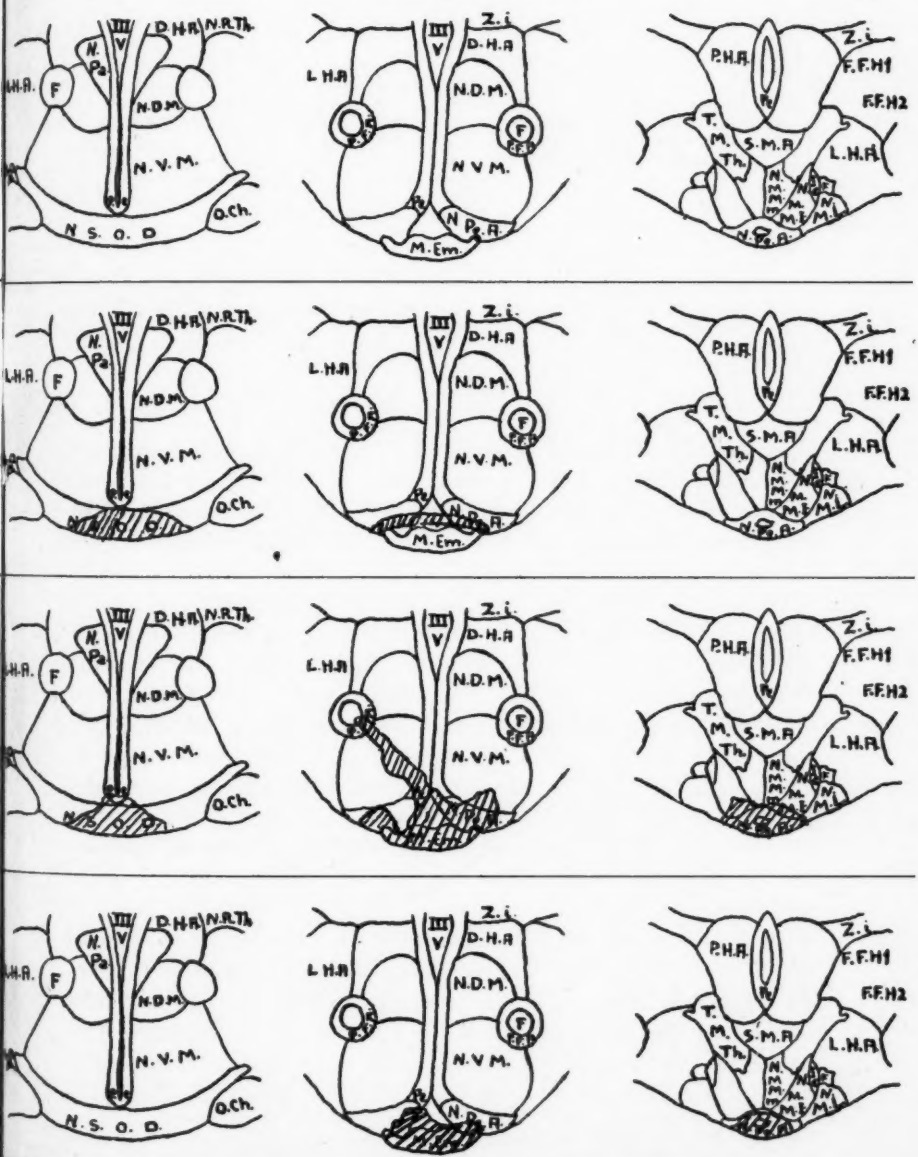


TABLE II-B (continued)

Dog no. and sex	Time after hypophysectomy	Post-mortem state of hypophysis	Hypothalamus
10 M	1 mo., 5 days	No pituitary tissue was found in the membranes attached to the external surface of the hypothalamus; sella turcica not examined	There is a defect in nerve substance in the middle of the floor of the 3rd ventricle including the ventro-medialis, periventricular arcuatus, and the inferior part of the walls of the 3rd ventricle. There is apparent diminution of cells of the supra-optic nuclei on comparison with normal.
11 M	10 days	No pituitary tissue was found in the membranes covering the external surface of hypothalamus; sella turcica not examined	Perivascular infiltration of lymphocytes and some plasma cells were found in different areas of the brain and meninges. There were also small, widely scattered areas of demyelinization. These findings were interpreted as evidence of meningo-encephalitis of undetermined etiology probably induced by operation.

fatty deposits. Pituitary remnants were found in 2 dogs surviving 6 and 8 months (dogs 9 and 8). In the other 2, surviving 10 days and 1 month, no pituitary remains were noted in the connective tissue covering the external surface of the hypothalamus, but the sella turcica was not studied in serial sections.

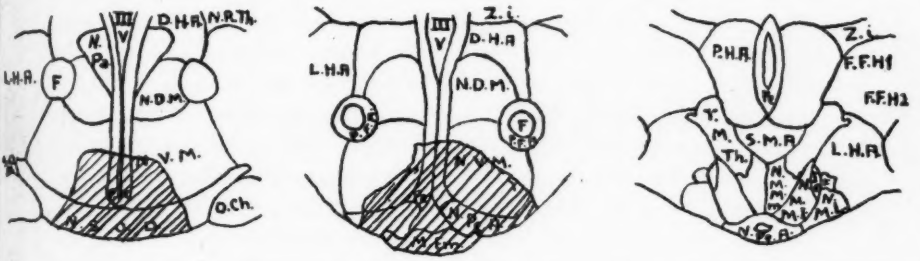
On analysis of the hypothalamic lesions (Table I-B) positive correlation is noted with respect to the occurrence of fatty cirrhosis and the finding of extensive defects of the hypothalamus (see schematic drawings and Figs. 9 to 12). The defects seem to be attributable to the operation. Lesions vary somewhat from case to case, but in each there were irregular defects of brain substance, with or without reaction, usually involving the nuclei * ventro-medialis on both sides, periventricularis arcuatus, and parts of surrounding hypothalamic structure. In all, the supra-optic nuclei showed reduction of the number of their cells independent of proximity to defects of the hypothalamus.

By comparison, the hypothalamic lesions were not nearly so extensive in the noncirrhotic dogs (Table II-B) with relatively normal livers. In dog 6, surviving 5¼ years, there was no defect except diminution in cells of the supra-optic nuclei—a change to be expected after transection of the pituitary stalk.[5] In dog 7, surviving almost 4 years, minimal hypothalamic injury was discerned consisting only of a micro-

* Terminology is that recommended in *The Hypothalamus. A. Research Nerv. & Ment. Dis., Proc.*, 1940, 20.

TABLE II-B (continued)

Schematic drawings* to show the approximate location of the lesions in the hypothalamus from coronal sections through the diencephalon. Drawings are made with right side depicted on reader's left.



Erosions could not be ascertained in this case because frozen sections were the only ones prepared and artifacts could not be avoided

*Abbreviations are as given in footnote to Table I-B.

scopic defect of the medial eminence. The other 4 dogs showed hypothalamic defects of different sizes; in 2 the defect was as large as in the cirrhotic groups. But these were not suitable for comparison since they survived only 10 and 35 days respectively. The other 2, dogs 9 and 8, surviving 6 and 8 months respectively, differed from the cirrhotic animals in that their hypothalamic defects were not so extensive (see schematic drawings). The only comparable defect in this group was found in dog 10 surviving 35 days. It had an apparently normal liver.

Adrenals

Inasmuch as atrophy of the liver seemed to be a part of the changes found, and cortical atrophy of the adrenal glands is generally accepted as a consequence of hypophysectomy,[6-9] the state of the adrenals was also correlated with the post-mortem condition of the hypophysis or its remains. Nine of the 11 dogs reported here showed mild to marked cortical atrophy of the adrenal glands; the 5 dogs with fatty cirrhosis were among these. The atrophy was apparently due to a reduction of the cortex and, particularly, to virtual disappearance of the cells of the reticular zone (Fig. 7). Since lipids remained in the intact cells, the reduction could not be attributed to its disappearance.

In 3 animals (dogs 3, 4 and 6) with varying degrees of adrenal atrophy, no pituitary remnants could be found upon examination of

serial sections through the sella turcica. One of these (dog 6) had had a unilateral adrenalectomy prior to hypophysectomy but the cortex of the surviving gland was regarded as slightly atrophic. The liver was normal. Two other dogs (dogs 2 and 7), with more marked adrenal atrophy, had microscopic nests of anterior pituitary cells attached to the meninges overlying the hypothalamus. In 1 dog the liver was not abnormal (dog 7), in the other it was cirrhotic (dog 2). Less marked adrenal cortical atrophy was noted in 2 other animals (dogs 1 and 9) and these had considerable residual pars tuberalis. In 2 additional dogs (dogs 5 and 8) with obviously smaller adrenal glands, microscopic pituitary remnants were found but the pars glandularis had been fully removed. The remaining 2 animals had normal adrenals but lived only 10 days and 1 month, respectively, after hypophysectomy.

In view of the inconstant atrophy of the adrenal glands, a correlation of this visceral change with the hypothalamic alterations was sought. This was further indicated because of the reports of splanchnomicria in human beings with hypothalamic injuries.[10] The 6 animals with marked adrenal atrophy (dogs 2, 3, 4 and 7, verified histologically, and dogs 5 and 8 with grossly contracted glands) had different degrees of hypothalamic defects. They were fairly extensive in all but 1 (dog 7). But dog 1, with much of the pars tuberalis left (Figs. 9 and 10) had only moderate adrenal cortical atrophy although it also had an extensive hypothalamic defect. The 2 dogs that survived 10 days and 1 month did not have narrowing of the adrenal cortex, although they had hypothalamic defects and no infundibular pituitary rests. (The sella was not examined microscopically for pituitary cells.)

The coexistence of hypothalamic defects and ablation of the pars glandularis makes it impossible to relate the reduction of adrenal cortex to one procedure alone. There may be differences in the nature and mode of reduction of the adrenal cortex following hypophysectomy as compared with changes following hypothalamic injury, but the material is not suitable to establish this point.

Thymus

In 7 dogs, 4 of the cirrhotic animals and 3 of the noncirrhotic, the thymus was easily found. In 3 it was regarded as hyperplastic, in others merely as not involuted. This finding likewise could not be related to hypophyseal reduction alone because of the coincidental hypothalamic damage in all but 1 (dog 6). This dog, surviving hypophysectomy 5 years with minimal hypothalamic change (*i.e.*, reduction of cells of the supra-optic nuclei), illustrates the "thymus-stimulating" effect of this operation.

Spleen

There was evidence of chronic congestion of the spleen in 4 of the cirrhotic animals; the organ was not saved in the fifth. Of the 4, three spleens were contracted and histologic examination disclosed atrophy of the white and red pulp.

Obesity

Because the cirrhotic animals had notable fat deposits in the liver, correlation with the occurrence of generalized obesity was made. The 2 dogs surviving hypophysectomy only 10 days and 35 days did not gain weight. In another animal (dog 9) weight changes were not recorded, but it did not appear obese at necropsy 6 months after hypophysectomy.

General obesity and marked increase of weight were observed in 8 dogs, including all 5 with fatty cirrhosis and dogs 6, 7 and 8; of these all but 1 (dog 6) had lesions in the hypothalamus. Of the other 7, 5 (dogs 1, 2, 5, 7 and 8) had microscopic clusters of anterior pituitary cells; 1 had most of the pars tuberalis. But dogs 3 and 4 also became obese and had had complete ablation of the hypophysis with defects of the hypothalamus. It is noteworthy that dog 6, surviving 5¼ years with complete hypophysectomy and no hypothalamic injury, also became obese. It should be noted that these animals were confined in cages or kennels with small runs for most of their postoperative period. Lowering of the body temperature and reduction of the metabolism [11-13] consequent to the partial or complete ablation of the pars glandularis * of the hypophysis or hypothalamic injury [14] may also have contributed to gain in weight. While it is notable that all 5 animals with fatty cirrhosis gained weight excessively, 3 other dogs with generalized obesity failed to show hepatic lesions excepting focal fatty change in 1.

Diabetes Insipidus

Lack of adequate clinical data precludes critical correlation and analysis of the time of occurrence, severity and duration of diabetes insipidus and the associated hypothalamic changes. However, no significant correlation could be found between diabetes insipidus and the occurrence of fatty cirrhosis. Diabetes insipidus was observed in 8 animals; it occurred with varying severity at different intervals after operation, and persisted for variable periods.

* Reports of the metabolic and thermal effects attributed to hypophysectomy alone have not been altogether satisfactory because of failure to examine or to record examination of the hypothalamus after the operation.

Discussion

When hepatic cirrhosis was first encountered among these dogs, consideration was given to possible etiologic factors unrelated to the hypophysectomy. Dietary influences were first excluded since no similar hepatic lesions have been encountered in a very large number of dogs kept on the same diet, in the same laboratories, for as long a time, but not subjected to hypophysectomy. Vermifuges containing possible hepatic toxins, such as carbon tetrachloride, were never employed. Bacterial or parasitic infections were not found. The increase of connective tissue has not been accompanied by leukocytic infiltration of any type, or by new capillary formation except in one instance (dog 4) and in this animal it was a chance finding.

However, it may be of interest to mention 1 other dog that we examined at necropsy through the courtesy of Drs. Shannon and Keller. Dr. Shannon has reported clinical studies [15] on this animal. It is not included in our tables because the pituitary gland was not removed, although transection of the pituitary stalk was performed together with destruction of a considerable area of contiguous hypothalamic tissue. The animal died 2 years after the operation and the only significant post-mortem visceral finding was diffuse suppurative and interstitial hepatitis, associated with wide zones of contraction and atrophy of the parenchyma. Only moderate, irregularly distributed deposits of lipid globules could be found in the intact liver cells. No inflammatory changes were found in the gallbladder, biliary passages, or the blood vessels. No organisms were demonstrated in the lesions. The lesions were classified as probably infectious in origin, although the possibility of subacute yellow atrophy has also been kept in mind. No operation other than the intracranial procedures had been done and the development of the severe hepatic lesions could not be related to any other events.

There seems little likelihood that the cirrhosis in the series of dogs reported here was spontaneous. In post-mortem examinations of about 300 dogs by one of us (I. G.) and of 3000 to 4000 dogs by another (I. H. P.), we have not encountered hepatic cirrhosis as a spontaneous lesion. In a personal communication, Dr. William Feldman of the Mayo Clinic stated that he has not found spontaneous hepatic cirrhosis in an equally large number of dogs. One of our animals had obvious cirrhosis only 4½ months after the intracranial operation and the time interval seems too short for the development of such an advanced lesion. Nevertheless, it had the most extensive hypothalamic defect in the series. The 5 animals with hepatic cirrhosis had, in common,

fatty deposits in the liver, adrenal cortical atrophy, involutional changes in the gonads, more or less complete hypophysectomy, and severe hypothalamic lesions. There were postoperative obesity, a change in their coats characterized by softness and luxuriant growth, and diabetes insipidus of varying severity. The absence of all or most of the anterior hypophysis may be held responsible for some of these changes but not for the hepatic lesion. We believe that the latter lesion can be more reasonably related to the hypothalamic defects.

Fat deposition in the liver after hypophysectomy was first attributed by Goetsch, Cushing and Jacobson [16] to the accompanying general disturbance in lipid metabolism. With the development of better experimental technic it was later appreciated that general obesity and genital atrophy could be induced by hypothalamic defects alone.[17, 18] Smith [6] has shown that obesity does not follow simple ablation of the pars glandularis of the hypophysis. Furthermore, Chaikoff, Gibbs, Holtom and Reichert [19] have reported that the hepatic lipids were in normal amounts up to 4 months after complete hypophysectomy in most of their dogs. However, one-third of them presented unexplained persistently high blood lipids. This point will receive further consideration later in this paper. In a study of the effects of hypophysectomy in puppies, Dandy and Reichert [20] reported cessation of growth, infantilism, and possible recession in size of the thyroid, sex glands and pancreas. They found no abnormalities in the livers of animals surviving as long as 2½ years. It is notable that none of their puppies became obese. Our own findings show that deposition of lipids in the liver is not inevitable after anterior hypophysectomy.

The early work attributing fatty deposit in the liver, like general adiposity, to the removal of the anterior lobe of the hypophysis must be qualified, therefore, by the knowledge that little or no attention was paid to the probable coexistence of injury to centers in the hypothalamus as an incidental result of the operation. Because of the short infundibular stalk, the extension of the tuber cinereum into it and its envelopment by the posterior, superior portion of the pars anterior, it is virtually impossible to perform an hypophysectomy in the dog without injuring the tuber cinereum (Van Dyke [21]). In our series only 1 dog (no. 6) escaped any detectable hypothalamic damage.

There is increasing evidence that fatty deposits in the liver may be related to hypothalamic lesions. Kraus [2, 3] has claimed that fatty deposits, usually pericentral in location, occur in the liver of man in association with certain neurologic disturbances, notably cerebral tumors accompanied by increased intracranial pressure, in Cushing's syndrome, and especially in patients with lesions of the hypothalamo-

hypophyseal pathways. It is well known that lesions in the region of the tuber cinereum (due to encephalitis, tumors, hydrocephalus) may cause the syndrome of pituitary obesity (Erdheim,[22, 23] Gottlieb,[24] Raab [25]).

One of us (I. G.) has recently encountered two instances illustrating this point. One was of mammary carcinoma in a middle-aged woman with widespread metastases, including the pars nervosa of the pituitary gland, extending well up the infundibular stalk. The pars glandularis was unaffected histologically. The patient had retained mild obesity and developed severe diabetes insipidus 6 weeks before death. The liver had moderate deposits of neutral fat in the central three-fifths of each otherwise normal lobule. The other instance has been reported by Collins.[26] Through his courtesy we studied the histologic preparations from the organs of a woman, 25 years old, who had died in coma after a long illness. At necropsy a huge astrocytoma was found replacing the hypothalamus but sparing the anterior hypophysis. The viscera were small (notably the adrenal), and the liver had fatty deposits affecting four-fifths of each otherwise normal lobule.

Brobeck, Tepperman and Long [27] have recently recorded supporting observations in rats with experimental hypothalamic lesions. The frequent occurrence of fatty livers in their animals was striking. In a personal communication Dr. Tepperman stated: "We would like to emphasize the fact that all of the animals showing high blood and liver lipids were obese, and that (although our series was rather small) there seems to be a rough correlation between the level of liver fat and the degree of obesity. We have made no liver lipid determinations on animals whose food intake was limited to that of their controls. This is an important omission and we hesitate to ascribe any of the metabolic abnormalities we have seen to the hypothalamic lesion itself. We believe that many of the phenomena may prove to be attributable to changes in food habit produced by the lesions." If this idea is correct, the occurrence of fatty infiltration of the liver in the presence of hypothalamic lesions may be found to be unrelated to any direct nervous or hormonal influence on the liver and this important suggestion may furnish a clue for resolving the conflicting evidence and views on this subject. In the 11 dogs reported here, lipid deposit in the liver was found more often in the cirrhotic animals then in the noncirrhotic. Minimal hypothalamic injury was observed in the latter and especially in those dogs surviving 5 and 3½ years. The absence of such fatty deposits may be evidence in support of this hypothesis.

The appearance of fat in the cirrhotic livers of dogs may be a reflection of the tendency of that substance to accumulate in the cirrhotic

organ. Or it may reflect a basic disturbance in which both fatty deposits and fibrosis are related or independent manifestations. From the data in the tables presented here it does not seem likely that generalized obesity is responsible for fatty infiltration of the liver in this series of animals. It is noteworthy that some obese animals did not have significant amounts of microscopically visible fat in the liver.

Another factor needs to be discussed, namely, the possibility that splanchnomicria may contribute to atrophy of the liver cords, whether fatty or not, with a relative increase of fibrous tissue or an actual replacement fibrosis. Unfortunately we did not have hepatic weights in all of our animals or a standard of reference for normal dogs. Nevertheless, in the animals with mild cirrhosis that had hypothalamic defects, the hepatic lobules appeared to be reduced in size and the trabeculae seemed narrower than normal. Observations on rats that have a bearing on this point have been recorded by Lee and Ayres.[28] They noted shrinkage and loss of weight of the liver following hypophysectomy. Whether this was due to simple ablation of the anterior portion of the pituitary gland or to lesions in the hypothalamus is unknown. The splanchnomicria of Simmond's disease has usually been attributed to insufficiency of the pars glandularis. Evidence has been collected [10] to show that the syndrome may occur with diencephalic lesions. The patient of Collins [26] is a case in point. In the presence of an intact anterior hypophysis there was marked atrophy of the thyroid, the adrenal glands, the ovaries and the endometrium.

A more closely allied disorder that reflects a neurohepatic relationship is seen in Wilson's disease (hepatolenticular degeneration). The explanation of the occurrence of cirrhosis of the liver in this disorder has not been forthcoming. Studies of the brain have shown that in the Wilsonian form the degeneration is limited to the globus pallidus. But degenerative processes in the lenticular nucleus may extend to the thalamus, the substantia nigra, the red nucleus and the cerebellum in the form called pseudosclerosis of Strümpell-Westphal. In both forms, because of direct connections by nerve fibers,[29] an effect on the hypothalamus may be expected even when it is not directly involved. It is well known that the hepatic disturbances begin after the onset of neurologic manifestations, even years after the first neurologic symptoms. Although microscopic deposits of fat globules are occasionally encountered in the liver cells, they have been apparently regarded as incidental findings.[30]

It is not yet possible to draw conclusions concerning the neurologic factors operating in our dogs. The hypothalamic injuries involved tissues with unknown functional relations, among them groups of cells

designated as "nuclei," which were partly or completely removed. Except for the generally accepted rôle of the supra-optic nuclei in water metabolism, there are few satisfactory data concerning the function of the other "nuclei," although injury or deficiency may evoke striking changes. Furthermore, the injuries were seldom sharply localized; usually they extended irregularly from the surface of the hypothalamus inward and laterally. Future studies based on localized lesions may throw more light on these problems.

The cause of the increase of connective tissue in the livers of our dogs is equally obscure. This cirrhosis is similar to that reported in depancreatized dogs by Chaikoff, Connor and Biskind.[31] Their animals developed fatty livers at variable periods after pancreatectomy. "In those kept for from 2.6 to 5.5 years upon an adequate diet and insulin, 8 of 16 developed more or less interlobular fibrosis of the livers. . . . In 4 animals this was so pronounced, both grossly and microscopically, that the picture of a well advanced portal cirrhosis of the liver was present. By the time this severe cirrhosis had occurred, the fat content of the livers had returned to normal and there was little histological evidence that a markedly fatty liver had preceded the fibrosis." These authors also could find no direct relationship between the interval after pancreatectomy and the degree of fibrous proliferation. One wonders why the other half of their animals failed to develop cirrhosis even though lipid in excess had been laid down in the liver. Incidentally, no pancreatic changes were found in our dogs. Connor [32] also considered that the fatty liver of the alcoholic subject is an important preliminary stage in the development of portal cirrhosis.

The observations of Brobeck, Tepperman and Long,[27] relating obesity and hepatic lipids to disturbances in food habits secondary to experimental hypothalamic injury, point anew to the need for more information on the composition of the diet of experimental animals. In the light of their work any metabolic disturbance attributed to selective hypothalamic or pituitary injury must be re-examined with regard to the animals' diets. Their observations may furnish a clue to the previously unexplained high blood lipids in the hypophysectomized dogs of Chaikoff, Gibbs, Holtom and Reichert.[19]

The deposition of fat in the liver is still the subject of too much controversy to permit a positive statement as to the mechanism operating in our animals. The occurrence of fatty liver after certain diets,[33-38] the augmentation of fatty deposits after pancreatectomy [31] and the reports of hepatic necrosis or fatty cirrhosis developing after diets low in protein and fat [39, 40] or low in protein and high in fat, as observed in rats [41] and dogs,[42-44] show how diverse diets may lead to superficially similar hepatic changes. The relation of hepatic necrosis or cirrhosis to

antecedent or concomitant fatty deposition remains unexplained. In a recent study of hypophysectomized rats given high fat or high carbohydrate diets, Samuels, Reinecke and Ball [45] found more body fat and less liver fat in the animals operated upon than in controls on the same caloric intake of a given diet. Lacking information of the exact composition of the diet of our dogs, we can only speculate as to possible deficiencies or excesses of individual constituents.

To recapitulate, it appears reasonable to conclude that hypophysectomy itself (or the operations on the kidneys in the animals with perinephritis) had little if anything to do with the hepatic lesions encountered in these dogs. The hypothalamic lesions disclosed by histologic examination seem to have a pivotal rôle in the hepatic changes. They were most extensive in the cirrhotic animals and were associated with the usual visceral effects encountered in hypothalamic injuries located in the same general vicinity. The adiposity of the cirrhotic animals reflected the metabolic changes, and in the light of the findings and suggestions of Brobeck, Tepperman and Long [27] they may be construed as evidence of the alteration of food habits of our dogs. We gave no especial attention to the diet of our animals. It may have provided a combination of fat and protein, with or without adequate proportions of essential amino acids or other substances likely to lead to fatty cirrhosis like that reported in dogs,[41, 42] rats [38, 39, 40, 44, 46] and rabbits [47] on improper diets. So-called "hospital scraps" were the principal sources of food in some of our dogs; Purina dog biscuits were given daily and meat three times weekly to most of them. It is possible that insufficient protein was given our animals. While it is noteworthy that a similar diet failed to induce fatty cirrhosis or fatty infiltration among the "control" animals, it seems reasonable to hypothesize that the cirrhotic dogs may have acquired fatty deposits because of excessive food intake, due to augmented appetites following the hypothalamic injuries.

Conclusions

1. Fatty cirrhosis of the liver was encountered in 5 dogs that had been hypophysectomized 4½ months to 3¾ years prior to post-mortem examination.

2. Six noncirrhotic animals examined 10 days to 5 years after hypophysectomy failed to exhibit fatty deposit or other hepatic change although moderate obesity was present in the animals surviving 8 months to 5 years.

3. Associated visceral and physiologic changes, such as adrenal cortical atrophy, thymic hyperplasia, coat changes, obesity, and diabetes insipidus, could not be related to the presence or absence of hepatic changes.

4. Study of the hypothalamus disclosed defects of variable position and extent in the cirrhotic animals that were invariably greater than in the control animals after hypophysectomy.

5. Consideration of the literature indicates that the variable reports of fatty infiltration of the liver after hypophysectomy did not take into account the rôle of associated, often unsuspected, hypothalamic injury. Furthermore, analysis of the results of uncomplicated hypophysectomy showed that obesity and fatty infiltration of the liver do not follow. In contrast, hypothalamic injury is regarded as probably responsible for the disturbance in metabolism and perhaps changes in food habits leading to obesity and fatty infiltration of the liver depending on the diet of the animal.

6. A hypothetical explanation is offered to account for fatty infiltration and cirrhosis of the liver based upon the unanticipated hypothalamic injury coincidental to hypophysectomy. Changes in food habits may have led to excessive ingestion of a ration providing unbalanced proportions of the various constituents like those shown to lead to fatty cirrhosis.

After this paper was submitted for publication, Chaikoff, Entenman, Rinehart and Reichert [48] reported the development of cirrhosis in the livers of dogs deprived of both pituitary and thyroid glands. They found high fat contents in the livers of the animals with hepatic cirrhosis. They did not observe the lesion in dogs that were subjected to thyroidectomy alone. They verified the completeness of hypophysectomy at necropsy, but made no mention of hypothalamic examination.

REFERENCES

1. Page, I. H., Graef, I., and Sweet, J. E. The development of hepatic cirrhosis following hypophysectomy. (Abstract.) *Am. J. Path.*, 1939, **15**, 647.
2. Kraus, E. J. Über Leberverfettung bei zerstörenden Prozessen im Hypophysenzwischenhirnsystem und bei Morbus Cushing. *Frankfurt. Ztschr. f. Path.*, 1936–37, **50**, 429–441.
3. Kraus, E. J. Chronischer Hirndruck und Leberverfettung. *Virchows Arch. f. path. Anat.*, 1937, **300**, 617–640.
4. Sweet, J. E. The hypophysis and body metabolism. *Ann. Surg.*, 1935, **102**, 1069–1073.

4a. Loyez, Marie. Coloration des fibres nerveuses par la méthode à l'hématoxyline au fer après inclusion à la celloïdine. *Compt. rend. Soc. de biol.*, 1910, **69**, 511–513.

5. Rasmussen, A. T. Effects of hypophysectomy and hypophysial stalk resection on the hypothalamic nuclei of animals and man. *A. Research Nerv. & Ment. Dis., Proc.*, 1940, **20**, 245–269.
6. Smith, P. E. The disabilities caused by hypophysectomy and their repair. The tuberal (hypothalamic) syndrome in the rat. *J. A. M. A.*, 1927, **88**, 158–161.
7. Houssay, B. A., and Sammartino, R. Modifications histologiques de la surrénale chez les Chiens hypophysoprives ou à tuber lésé. *Compt. rend. Soc. de biol.*, 1933, **114**, 717–721.
8. Houssay, B. A., Biasotti, A., and Mazzocco, P. Les poids des surrénales des Chiens hypophysoprives ou à tuber lésé. *Compt. rend. Soc. de. biol.*, 1933, **114**, 714–716.

9. Van Dyke, H. B. The Physiology and Pharmacology of the Pituitary Body. University of Chicago Press, Chicago, 1936, **1**, pp. 38, 57, 64 and 70.
10. Gagel, O. Symptomatologie der Erkrankungen des Hypothalamus. In: Bumke, O., and Foerster, O. Handbuch der Neurologie. J. Springer, Berlin, 1936, **5**, 506–513.
11. Benedict, F. G., and Homans, J. The metabolism of the hypophysectomized dog. *J. M. Research*, 1911–12, **25**, 409–502.
12. Aschner, B., and Porges, O. Über den respiratorischen Stoffwechsel hypophysipriver Tiere. *Biochem. Ztschr.*, 1912, **39**, 200–204.
13. Houssay, B. A. The influence of the pituitary on basal metabolism and on specific dynamic action. *Endocrinology*, 1934, **18**, 409–414.
14. Ranson, S. W. Regulation of body temperature. *A. Research Nerv. & Ment. Dis., Proc.*, 1940, **20**, 342–399.
15. Shannon, J. A. The control of the renal excretion of water. I. The effect of variations in the state of hydration on water excretion in dogs with diabetes insipidus. *J. Exper. Med.*, 1942, **76**, 371–386.
16. Goetsch, E., Cushing, H., and Jacobson, C. Carbohydrate tolerance and the posterior lobe of the hypophysis cerebri. An experimental clinical study. *Bull. Johns Hopkins Hosp.*, 1911, **22**, 165–190.
17. Camus, J., and Roussy, G. Les syndromes hypophysaires. Anatomie et physiologie pathologiques. *Rev. neurol.*, 1922, **38**, 622–639.
18. Bailey, P., and Bremer, F. Experimental diabetes insipidus. *Arch. Int. Med.*, 1921, **28**, 773–803.
19. Chaikoff, I. L., Gibbs, G. E., Holtom, G. F., and Reichert, F. L. The lipid metabolism of the hypophysectomized dog and the lipid and carbohydrate metabolism of the hypophysectomized-depancreatized dog. *Am. J. Physiol.*, 1936, **116**, 543–550.
20. Dandy, W. E., and Reichert, F. L. Studies on experimental hypophysectomy in dogs. III. Somatic, mental and glandular effects. *Bull. Johns Hopkins Hosp.*, 1938, **62**, 122–155.
21. Van Dyke, H. B. The Physiology and Pharmacology of the Pituitary Body. University of Chicago Press, Chicago, 1936, **1**, 34.
22. Erdheim, J. Über Hypophysenganggeschwülste und Hirncholesteatome. *Sitzungs. d. k. Akad. d. Wissensch. Math.-naturw. Cl.*, 1904, **113**, pt. 3, 537–726.
23. Erdheim, J. Nanosomia pituitaria. *Beitr. z. path. Anat. u. z. allg. Path.*, 1916, **62**, 302–377.
24. Gottlieb, K. Die Pathologie der Dystrophia adiposogenitalis. *Ergebn. d. allg. Path. u. path. Anat.*, 1921, **19**, pt. 2, 575–649.
25. Raab, W. Klinische und röntgenologische Beiträge zur hypophysären und zerebralen Fettsucht und Genitalatrophie. *Wien. Arch. f. inn. Med.*, 1924, **7**, 443–530.
26. Collins, V. P. Effects of destruction of hypothalamus by tumor. *Arch. Neurol. & Psychiat.*, 1942, **48**, 774–788.
27. Brobeck, J. R., Tepperman, J., and Long, C. N. H. Further observations on experimental hypothalamic obesity. *Proc. Fed. Am. Socs. Exper. Biol.*, 1942, **1**, pt. 2, 10–11.
28. Lee, M., and Ayres, G. B. The composition of weight loss and the nitrogen partition of tissues in rats after hypophysectomy. *Endocrinology*, 1936, **20**, 489–495.
29. Ingram, W. R. Nuclear organization and chief connections of the primate hypothalamus. *A. Research Nerv. & Ment. Dis., Proc.*, 1940, **20**, 195–244.
30. Hall, H. C. La dégénérescence hépato-lenticulaire. Masson et Cie., Paris, 1921.
31. Chaikoff, I. L., Connor, C. L., and Biskind, G. R. Fatty infiltration and cirrhosis of the liver in depancreatized dogs maintained with insulin. *Am. J. Path.*, 1938, **14**, 101–110.

32. Connor, C. L. The etiology and pathogenesis of alcoholic cirrhosis of the liver. *J. A. M. A.*, 1939, **112**, 387–390.
33. Best, C. H. The rôle of the liver in the metabolism of carbohydrate and fat. *Lancet*, 1934, **1**, 1274–1277.
34. Best, C. H. The significance of choline as a dietary factor. *Science*, 1941, **94**, 523–527.
35. Channon, H. J., and Wilkinson, H. The effect of various fats in the production of dietary fatty livers. *Biochem. J.*, 1936, **30**, 1033–1039.
36. Channon, H. J., Loach, J. V., Loizides, P. A., Manifold, M. C., and Soliman, G. The effect of proteins in the prevention of dietary fatty livers. *Biochem. J.*, 1938, **32**, 976–985.
37. Beeston, A. W., and Channon, H. J. Cystine and the dietary production of fatty livers. *Biochem. J.*, 1936, **30**, 280–284.
38. Gavin, G., and McHenry, E. W. The B vitamins and fat metabolism. III. The effects of B_6 upon liver and body fat. *J. Biol. Chem.*, 1940, **132**, 41–46.
39. Lillie, R. D., Daft, F. S., and Sebrell, W. H., Jr. Cirrhosis of the liver in rats on a deficient diet and the effect of alcohol. *Pub. Health Rep.*, 1941, **56**, 1255–1258.
40. Lillie, R. D., Ashburn, L. L., Sebrell, W. H., Jr., Daft., F. S., and Lowry, J. V. Histogenesis and repair of the hepatic cirrhosis in rats produced on low protein diets and preventable with choline. *Pub. Health Rep.*, 1942, **57**, 502–508.
41. Blumberg, H., and Grady, H. G. Production of cirrhosis of the liver in rats by feeding low protein, high fat diets. *Arch. Path.*, 1942, **34**, 1035–1041.
42. Chaikoff, I. L., and Connor, C. L. Production of cirrhosis of the liver of the normal dog by high fat diets. *Proc. Soc. Exper. Biol. & Med.*, 1940, **43**, 638–641.
43. Chaikoff, I. L., Eichorn, K. B., Connor, C. L., and Entenman, C. The production of cirrhosis in the liver of the normal dog by prolonged feeding of a high-fat diet. *Am. J. Path.*, 1943, **19**, 9–21.
44. Fouts, P. J. Vitamin B complex studies in dogs. Production of cirrhosis of liver. *J. Nutrition*, 1943, **25**, 217–228.
45. Samuels, L. T., Reinecke, R. M., and Ball, H. A. Liver fats and glycogen of hypophysectomized rats on high carbohydrate and high fat diets. *Proc. Soc. Exper. Biol. & Med.*, 1942, **49**, 456–458.
46. György, P., and Goldblatt, H. Hepatic injury on a nutritional basis in rats. *J. Exper. Med.*, 1939, **70**, 185–192.
47. Rich, A. R., and Hamilton, J. D. The experimental production of cirrhosis of the liver by means of a deficient diet. *Bull. Johns Hopkins Hosp.*, 1940, **66**, 185–198.
48. Chaikoff, I. L., Entenman, C., Rinehart, J. F., and Reichert, F. L. Development of cirrhosis in the liver of dogs deprived of both pituitary and thyroid glands. *Proc. Soc. Exper. Biol. & Med.*, 1943, **54**, 170–171.

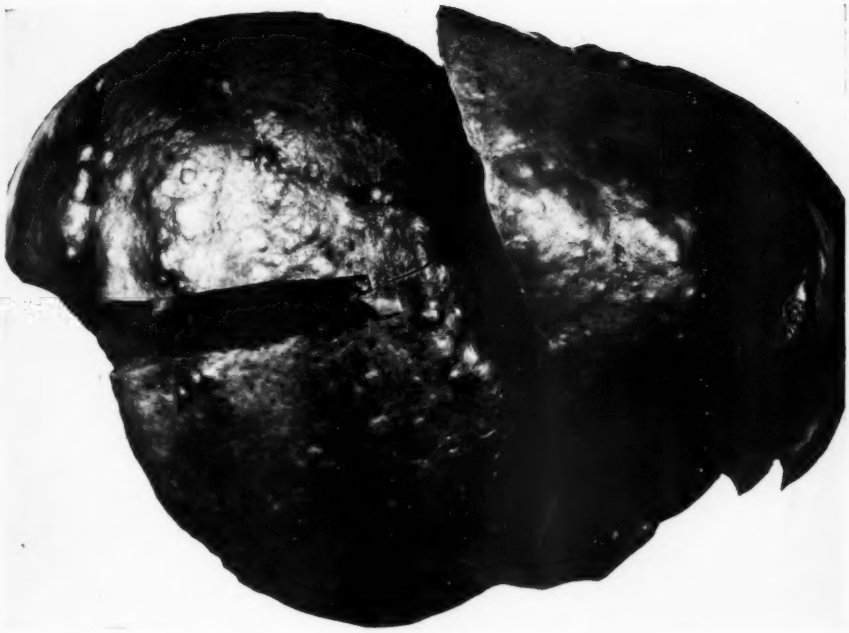
DESCRIPTION OF PLATES

PLATE 152

FIG. 1. The nodular appearance of the liver in dog 2.

FIG. 2. Low-power photomicrograph of the liver shown in Figure 1, demonstrating the multilobular deposits of connective tissue. There are numerous hypertrophied areas as well as atrophic zones. (See Fig. 6 for higher magnification.) × 10.

1



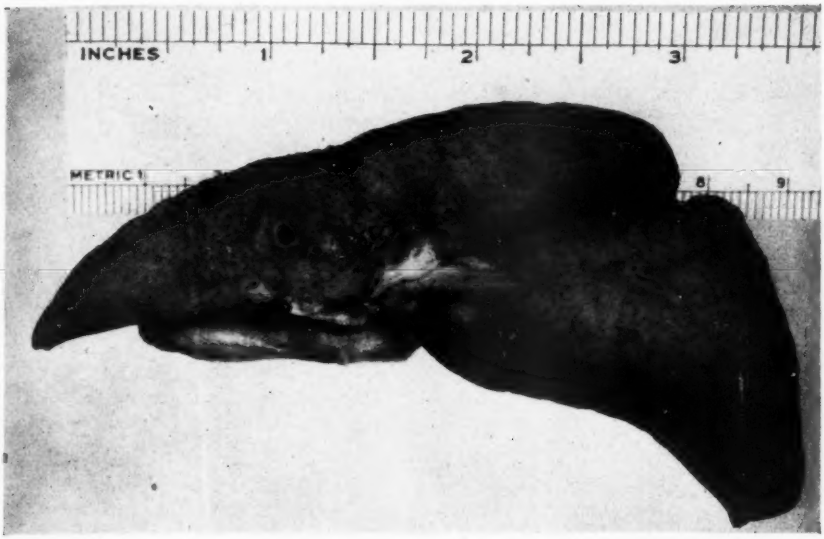
2

Graef, Negrin and Page Hepatic Cirrhosis after Hypophysectomy

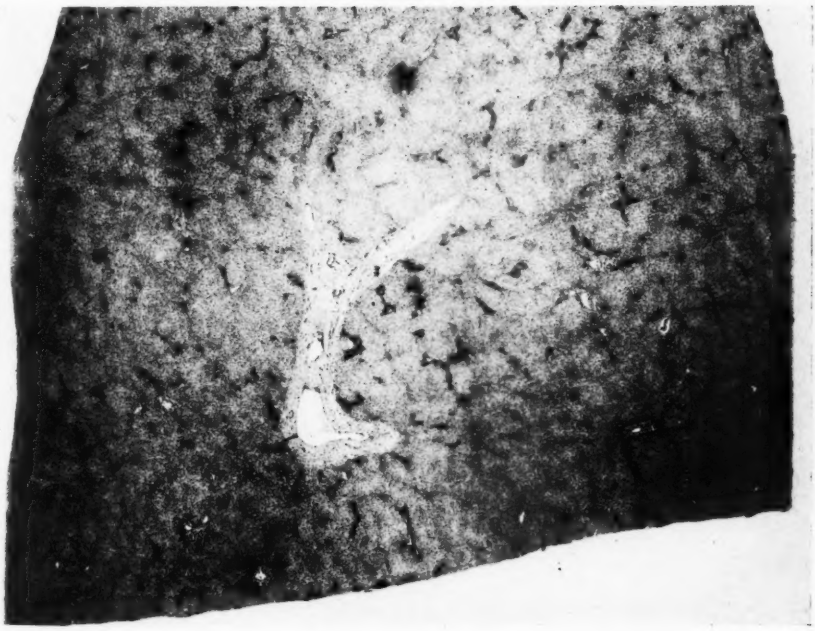
PLATE 153

FIG. 3. Photograph of the cut surface of the liver from dog 4, illustrating the prominent appearance of the lobular markings. (See Figs. 4 and 5 for photomicrographs.)

FIG. 4. Low-power photomicrograph of a section of liver shown in Figure 3. The dark areas are prominent portal zones infiltrated with lymphocytes and connective tissue. (See Fig. 5 for higher magnification.) × 12.



3



4

Graef, Negrin and Page Hepatic Cirrhosis after Hypophysectomy

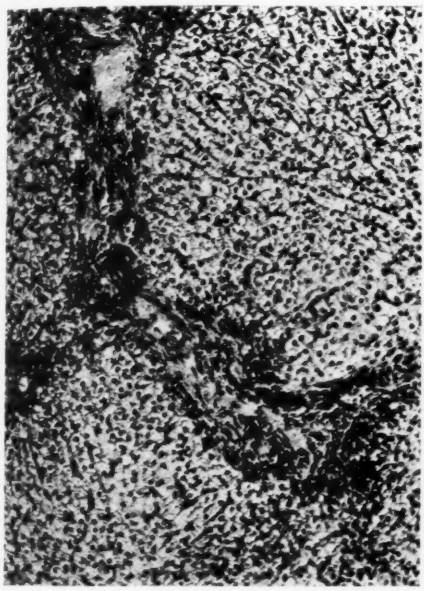
PLATE 154

FIG. 5. Photomicrograph of a section of the liver of dog 4, showing fatty vacuoles in the hepatic trabeculae. Periportal and perilobular fibrosis are also shown. Hematoxylin and eosin stain. × 105.

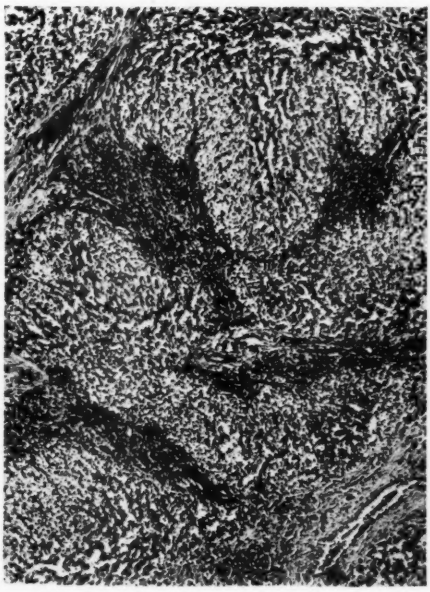
FIG. 6. Section of liver shown in Figure 1 taken through an area of marked fibrosis and atrophy of the liver parenchyma. Some trabeculae show marked fatty vacuolization; others show marked narrowing with widening of the hepatic sinusoids. Hematoxylin and eosin stain. × 79.

FIG. 7. Section of the adrenal gland from dog 4, showing severe cortical atrophy. The medulla occupies the greater part of the field. The cortico-medullary junction extends along the line from A to A'. Hematoxylin and eosin stain. × 52.

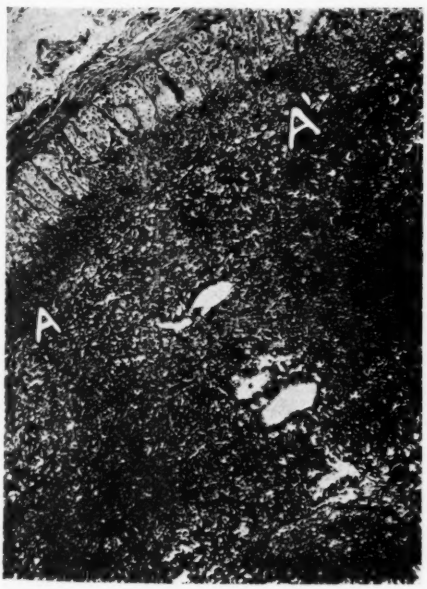
FIG. 8. Section of the adrenal gland from dog 6. The animal survived hypophysectomy and unilateral adrenalectomy 5¼ years. The glomerular zone is intact. The fascicular and reticular zones appear intact although moderately depleted of lipid. Hematoxylin and eosin stain. × 52.



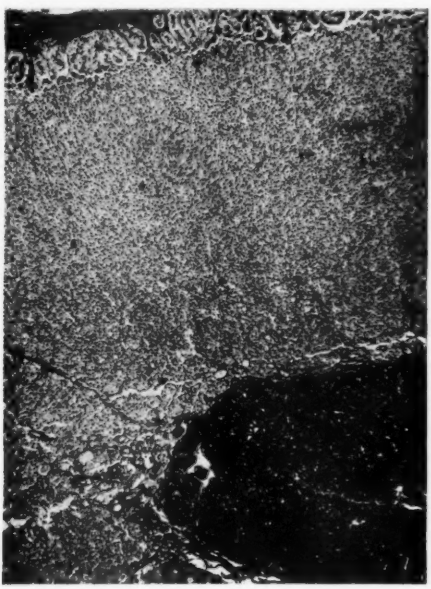
5



6



7



8

Graef, Negrin and Page

Hepatic Cirrhosis after Hypophysectomy

PLATE 155

FIG. 9. Coronal section through hypothalamus of dog 1 at the level of the median eminence, showing rests of pars tuberalis (A) attached to it and a defect of nervous tissue in the medial part of the n. ventro-medialis (B). Hematoxylin and eosin stain. × 60.

FIG. 10. In this photomicrograph from dog 1, the remnants of the pars tuberalis (A) are seen attached to the median eminence whose lateral part is missing (B). It forms part of a lesion consisting of a defect of the hypothalamus produced presumably when the animal was hypophysectomized 2½ years before death. Hematoxylin and eosin stain. × 55.

FIG. 11. Coronal section through hypothalamus of dog 2 at the level of the median eminence. The latter is replaced almost completely by connective tissue scar (A) growing from the meningeal membranes (B) covering the inferior part of this portion of the diencephalon. A defect (C) includes the n. periventricularis arcuatus and medial portion of the n. ventro-medialis on both sides; deposits of calcium salts appear as black spots. Hematoxylin and eosin stain. × 55.

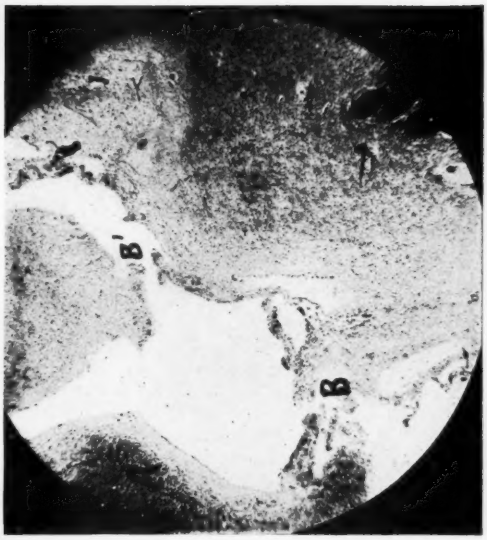
FIG. 12. Sagittal section through hypothalamus of dog 5. On the left can be seen the myelin of the fibers of the optic chiasma (A); on the right side an arrow points to the myelin of the fornix. A defect of nervous tissue is noted between both structures (at B and B′) involving the n. ventro-medialis in this photomicrograph. Combined myelin and Nissl stain. × 60.

10

9

Graef, Negrin and Page

12



11

Hepatic Cirrhosis after Hypophysectomy

THE INFLUENCE OF AN ACTIVATED STEROL ON BLOOD PRESSURE IN DOGS *

R. G. Mrazek, A.B., R. J. Jensik, M.D.,† and C. I. Reed, Ph.D.
(From the Department of Physiology, University of Illinois Chicago Colleges, Chicago, Ill.)

Claims of the hypertensive action of vitamin D have appeared persistently in the literature for many years, despite the absence of data that would support such claims. The only support for these claims lies in papers by Appelrot[1] and by Goormaghtigh and Handovsky[2] showing that in the dog apparently a fair degree of hypertension may be developed in response to vitamin D.

We have previously discussed this problem in some detail and have included data indicating that human subjects have more commonly responded by lowering of systolic blood pressure.[3] In no case could it be said that a significant hypertension had occurred.

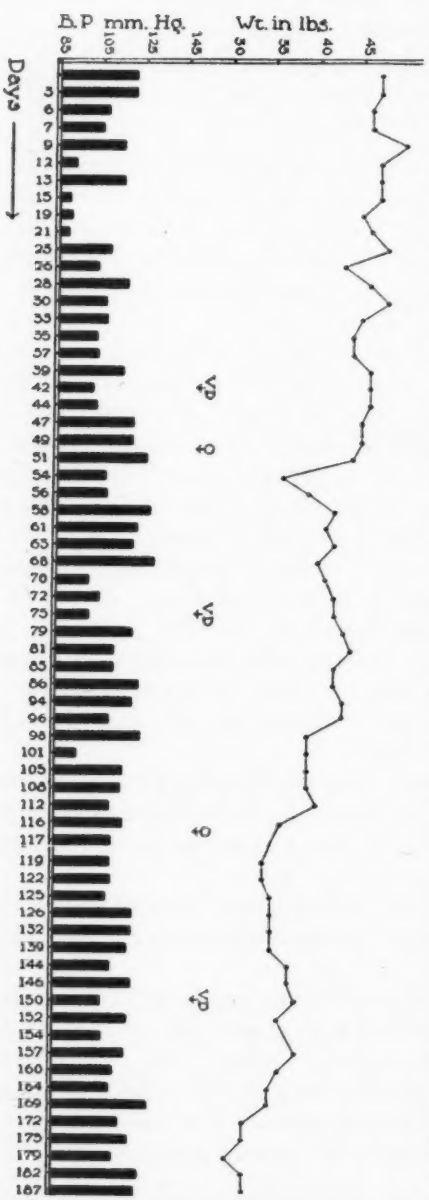
Recently, Briskin, Stokes, Reed and Mrazek[4] showed conclusively that no level of administration of vitamin D would produce hypertension in albino rats. Nevertheless, the results on dogs[1,2] were concise and in spite of numerous earlier failures to produce hypertension in this species by means of vitamin D, it was decided to reopen the problem, using the intra-arterial method of recording pressures. A terminal check under nembutal anesthesia with simultaneous readings on the dial recorder connected with the needle in an artery and on a mercury manometer attached to an arterial cannula showed good agreement between the two methods.

The methods of training have been described previously and no new features of significance were introduced. Preliminary control readings were taken at intervals of 1 to 5 days over a period of 1 month to 6 weeks.

At frequent intervals, sensitivity to adrenalin was tested on 8 dogs in view of the reported observation of increased sensitivity to adrenalin induced by vitamin D.

Twenty dogs were used, 12 being under observation for periods ranging from 108 to 187 days; the other 8 were observed for 38 to 73 days. A protocol is presented in Table I and Text-figure 1. The body weight during the control period of 41 days was maintained at a fairly constant level. With training, the mean arterial pressure was reduced slightly. The average for 19 readings in the entire training period was 106 mm.

* Supported in part by grants from the Graduate School Research Board and from the Nutrition Research Laboratories.
Received for publication, December 2, 1943.
† Department of Pathology.



Text-Fig. 1. Graphic presentation of the data given in Table I.

Hg. Starting with the 42nd day of observation, the dogs received daily 200,000 units of vitamin D as ertron for 8 days, or approximately 10,000 units per kg. per day. With the appearance of anorexia, administration was discontinued. Within the next few days weight declined sharply and was never again recovered completely.

While there were high readings of mean blood pressure following this period, the mean for the entire period of 44 to 75 days inclusive was 114 mm.

At this time a second period of administration was begun and continued through the 116th day, resulting in further weight loss. Except for minor differences, the mean intra-arterial pressure maintained at a slightly lower level. The mean of 12 readings during this period was again 114 mm.

After a rest period, administration of the vitamin was resumed on the 150th day and continued through the 172nd day. The mean of seven readings was 116 mm., and for four subsequent readings, 118 mm. In view of the large variations it cannot be considered that the progressively ascending mean for each successive period represents hypertension.

In all of the animals isolated, extremely high readings occurred in both control and experimental periods, as well as occasional extremely low ones. These could seldom be correlated with any demonstrable change in the animal's condition or environment. In fact, the same animal might progress from one extreme to the other between two different readings made on the same day, although there was seldom any pronounced change observable during the time that the needle was in place, except for a slight decrease after the initial puncture was made.

The extensive utilization of this method of testing blood pressure in intact dogs in this department in recent years has produced sufficient data to justify the conclusion that all of the fluctuations observed in this study are within the physiological range (Wakerlin).[5]

Furthermore, the upward trend observed in this particular animal was reversed in five others (Table I). In the remainder there was practically an even mean pressure throughout. The tendency for blood pressure in rats to follow weight changes (Briskin and co-workers[4]) is not in evidence in the dogs of this series. This suggests that the two species possess different mechanisms of circulatory response to weight changes. In this respect the dog behaves more nearly like man.

The results of adrenalin tests likewise failed to confirm the claims of Goormaghtigh and Handovsky[2] that vitamin D sensitizes animals to this agent. In Text-figure 2 are shown two sets of graphs of eight adrenalin tests, the lower four during a period when no vitamin was given, the upper four during a period of daily administration of 200,000 units. Graphs for the adrenalin tests on the other dogs showed just as little difference as in this illustration. It is apparent, then, that vitamin D does not sensitize dogs to adrenalin. The standard dose of 1.5 gamma per kg. was established by preliminary trial as an approximate threshold dose, since it seemed probable that any sensitization would be more readily apparent at a moderate level.

Just as this manuscript was being prepared, Katz, Rodbard and Meyer[6] published a report of the administration of vitamin D_2 in propylene glycol to 10 dogs, 5 of which were experimental renal hypertensive animals, 3 were spontaneously hypertensive and 2 were normotensive. Subcutaneous injections of 40,000 units per day for 31 days to the 3 spontaneous hypertensive dogs and oral administration of 400,000 units per day to the others produced no evidence of hypertensive response except in 3 animals. In 1 normotensive dog with denervation of the carotid sinus there was mean elevation by 24 mm. Hg systolic/16 mm. Hg diastolic during 15 days, which disappeared thereafter. A similar reaction occurred in 1 spontaneous hypertensive dog with a

TABLE I

Sample Protocol for 1 of the 20 Dogs Used

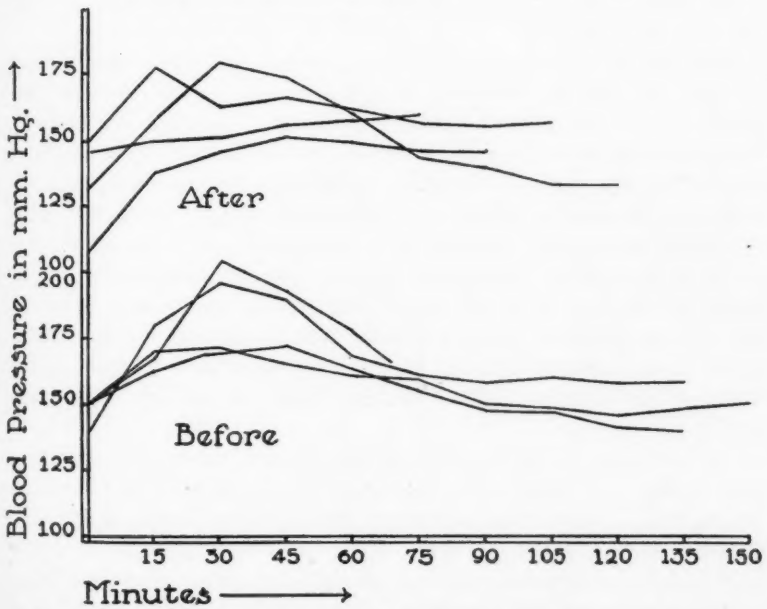
Days	Mean arterial pressure	Weight	Experimental procedure
	mm.Hg	*lbs.*	
	119	47	Control
3	119	47	
6	107	46	
7	105	46	
9	116	50	
12	93	47	
13	115	47	
15	90	47	
19	91	45	
21	90	46	
23	109	48	
26	103	43	
28	117	46	
30	107	48	
33	108	45	
35	102	44	
37	103	44	
39	109	46	
42	101	46	200,000 units of vitamin D
43			200,000 units of vitamin D
44	103	46	200,000 units of vitamin D
45		45	200,000 units of vitamin D
46			200,000 units of vitamin D
47	119	45	200,000 units of vitamin D
48		42	200,000 units of vitamin D
49	119	45	200,000 units of vitamin D
50			(anorexia)
51	126	44	(anorexia)
54	107	36	
56	108	39	
58	128	42	
61	122	41	
63	120	42	
68	130	40	
70	100	41	
72	105	42	
75	100	42	200,000 units, ertron
76	...	..	100,000 units daily through 108th day
79	119	43	*vide supra*
81	111	44	*vide supra*
83	112	42	*vide supra*
86	123	42	*vide supra*
94	119	43	
96	111	43	
98	124	39	*vide supra* (toxic)
101	95	39	*vide supra*
105	116	39	*vide supra*
108	115	39	*vide supra*
111	...	..	300,000 units
112	110	40	200,000 units
116	116	36	200,000 units
117	111	..	(anorexia)
119	111	34	(anorexia)
122	111	34	(anorexia)
125	109	35	Adrenalin test
126	121	35	Adrenalin test
132	121	35	
139	119	35	
144	111	37	Adrenalin test
146	121	37	Adrenalin test

TABLE 1 (continued)
Sample Protocol for 1 of the 20 Dogs Used

Days	Mean arterial pressure	Weight	Experimental procedure
	mm.Hg	*lbs.*	
150	107	38	100,000 units, ertron, daily through 168th day
152	119	36	*vide supra*
154	107	..	*vide supra*
157	118	38	*vide supra*
160	113	36	*vide supra*
164	111	35	*vide supra*
169	129	35	200,000 units daily
172	116	32	300,000 units daily
175	119	32	*vide supra*
179	113	30	Adrenalin test
182	121	32	*vide supra*
187	119	32	Adrenalin test

mean elevation of 19/18 mm. In 1 renal hypertensive animal a mean increase of 26/27 mm. was attained, and during the succeeding 15 days after administration the mean elevation was 27/35 mm.

The interpretation of these results would appear to us to depend on individual opinion, since neither the actual levels, the individual readings, nor the standard deviations were given. In our experience, if control observations are continued long enough, fully as large variations may occur in the control periods.



Text-Fig. 2. Adrenalin tests before and after the administration of activated sterol.

Another criticism lies in the failure to grade the dosage of vitamin D according to body weight. If this were done, it is not apparent from the report. However, the ultimate conclusion of these authors is that vitamin D has only a slight and irregular tendency to produce hypertension in dogs.

One might raise the question as to whether the differences are related to the particular preparation of vitamin D. This seems unlikely as there is, at present, no suggestion of evidence that any vehicle for this vitamin has hypertensive action, either alone or in combination with the vitamin. We have quite generally found ertron less toxic than other forms of vitamin D. The fact that ertron produced toxication but did not produce significant hypertension strongly suggests that hypertension is not a fundamental part of the syndrome of toxication by vitamin D in the rat, dog, or man. Katz, Rodbard and Meyer [6] supported that thesis for the dog. At present there is no evidence that ertron is less hypertensive than other forms of vitamin D merely because it is slightly less toxic. We have some evidence that the active material in ertron is not calciferol, while that used by Katz, Rodbard and Meyer is crystalline calciferol dissolved in propylene glycol. On the basis of present information there is no reason to assume that the chemical differences among the numerous vitamers D are of sufficient physiological significance to account for the apparent differences among the published reports.

Also, the form of vitamin used in this investigation produced weight loss and anorexia in some cases at levels as low as 5000 units per kg. per day. In every case the evidence indicates that the condition was true toxication by vitamin D since all animals recovered when administration was discontinued. When administration was continued uninterruptedly, the animals invariably died without developing hypertension. Therefore, it may be assumed that hypertension is not a part of the symptom complex of vitamin D-toxicity. Since it has occurred in the experience of others, it must have been due to some other factor or factors not apparent in the published reports and not measurable in terms of antirachitic potency. Also, it may be pointed out that Katz, Rodbard and Meyer [6] did not continue their observations as long as in this investigation.

Our previous attempts to demonstrate the development of hypertension in dogs with viosterol, crystalline calciferol, calciferol in propylene glycol and vitamin D_3 have proved unsuccessful as in the experiments reported here.

It should be stressed again that our experiments were of the following types: (1) long-term, moderate dosage; (2) very large individual

doses; (3) long-term, low dosage; (4) short-term, high dosage. Quantitatively and qualitatively the results were homogeneous among these types of experiments.

In an earlier experiment 1 dog was observed for 18 months after a period of toxicity in which the weight loss amounted to 50 per cent. The animal was estimated to be 8 years old. With the additional period of observation he was 9½ years old when the final blood pressure test was made. This should be comparable to 60 years or more in man; yet no hypertension was present, although some kidney damage was found.

TABLE II

Degree of Renal Arteriolar Hypertrophy as Related to Other Experimental Data

Dog no.	Weight	Mean blood pressure			Degree of medial hypertrophy in kidney	Vitamin D units		
		Before	After	Change		Total dose	Total per kg.	Dose per kg. per day
	kg.	*mm.Hg*	*mm.Hg*	*mm.Hg*				
11	12.7	116	114	− 2	+++	3,835,000	301,960	9,150
12	13.2	134	147	+13	+	6,030,000	456,820	13,052
13	14	134	135	+ 1	++	4,575,000	326,786	27,232
14	11.3	91	111	+20	++	4,245,000	375,664	13,416
35	11	131	125	−14	++	1,705,000	144,492	10,320
61	16.3	114	114	0	++	3,810,000	233,742	19,478
62	14.5	122	122	0	+	3,230,000	222,758	13,922
69	15.5	117	131	+14	+++	3,450,000	222,580	5,429

Tissues were removed from the last 8 dogs of the series for histopathological examination. In the myocardial arterioles there was occasionally seen a mild degree of medial hypertrophy. A similar change was seen less frequently in the arterioles of the spleen. It was only in the sections of the kidneys that any marked changes were found, in the form of varying degrees of arteriolar medial hypertrophy, cuneate areas of fibrosis, and lymphocytic infiltration. Since it seems probable that only the first of these changes is of possible significance in relation to blood pressure, further discussion will be confined thereto. A condensed arrangement of the data is presented in Table II.

The degree of pathological change is somewhat arbitrarily designated as 1 plus (+), 2 plus (++), or 3 plus (+++), the first representing the mildest state at which definite changes could be detected, the latter the most extreme degrees, approaching complete obliteration of the lumina. The question of the statistical significance of the changes in blood pressure has already been discussed. Nevertheless, the means of the pre-treatment and treatment periods show that there was a marked increase in 3 dogs, a marked decrease in 1, with no marked change in the other 4.

Rearrangement of the data in ascending order for each of the factors in Table II resulted in a wholly random distribution and complete failure to demonstrate a close correlation between the degree of the vascular lesion and any one of the factors in the other columns. Nevertheless, it is a fact that all of the dogs did show pathological changes in the renal arterioles and all of them received vitamin D.

The dog receiving the highest total dose, no. 12, showed only a first degree change, while dogs 11 and 69 showed third degree changes on moderate total doses, but low daily doses per kg. Paradoxically, dog 35 showed second degree changes with the lowest total dose.

Only 1 of the 3 dogs with high mean pressure after treatment showed third degree changes in the arterioles.

Conclusion

In a series of 20 dogs receiving doses of vitamin D of sufficient potency to produce toxicity manifested by anorexia and weight loss, blood pressure determinations by the intra-arterial needle method showed that hypertension had not been produced. Also standardized adrenalin tests showed that none of the dogs had developed sensitivity to this drug.

Microscopical examination of the kidneys of 8 of these dogs showed varying degrees of medial hypertrophy in the renal arterioles of all of them, but there was no close correlation between dosage, blood pressure changes and the degree of pathological change.

REFERENCES

1. Appelrot, S. Hypervitaminosis D and blood pressure in dogs. *Am. J. Physiol.*, 1933, **105**, 294–299.
2. Goormaghtigh, N., and Handovsky, H. Effect of vitamin D_2 (calciferol) on the dog. *Arch. Path.*, 1938, **26**, 1144–1182.
3. Reed, C. I., Struck, H. C., and Steck, I. E. Vitamin D. University of Chicago Press, Chicago, 1939.
4. Briskin, H. L., Stokes, F. R., Reed, C. I., and Mrazek, R. G. The effects of vitamin D and other sterols on blood pressure in the rat. *Am. J. Physiol.*, 1943, **138**, 385–390.
5. Wakerlin, G. E. Personal communication, 1943.
6. Katz, L. N., Rodbard, S., and Meyer, J. Blood pressure responses of dogs to vitamin A and vitamin D_2. *Am. J. Physiol.*, 1943, **140**, 226–229.

FEMINIZATION IN MALE DOGS

A Syndrome Associated with Carcinoma of the Testis and Mimicked by the Administration of Estrogens *

R. M. Mulligan, M.D.

(From the Department of Pathology, University of Colorado, School of Medicine, Denver, Colo.)

In human pathology several syndromes occurring with neoplasms of endocrine glands are well recognized. The coincidence of symptom-complexes and endocrine tumors in animals commonly employed experimentally not only offers an opportunity for the investigation of the pathologic alterations in the observed animal, but also affords some insight into similar diseases in man.

A review of the literature on canine neoplasms disclosed 6 cases of carcinoma of the testis with which a characteristic group of symptoms and signs were associated. When these cases were analyzed in relation to recent experiments in which diethylstilbestrol was administered to male dogs, the similarity of several findings in the experimental animals to the features of the clinicopathologic picture seen with carcinoma of the canine testis became evident. The clinical and pathologic details of these 6 cases of carcinoma of the testis, and the changes evoked in male dogs by the administration of natural and synthetic estrogens in the experience of other workers, will be compared and discussed together with recent experiments in which diethylstilbestrol was fed to male dogs.

Summary of 6 Cases of Carcinoma of the Testis from the Literature

In 1908, Wooldridge [1] described an aged black Pomeranian dog weighing 16 lbs., which for 1 year had experienced gradual enlargement of the right testis and simultaneous loss of hair which involved progressively the neck, abdomen, back and legs. During life the dog licked the enlarged right testicle and, in the words of the owner, "was pleased when we gave it a gentle rubbing." Two photographs illustrated the enlarged right testis and the general lack of hair. Also suggested in them was the prominence of the penile sheath and mammary glands. At autopsy the right testicle revealed a 17 oz. tumor, diagnosed as carcinoma by Sir John McFadyean. No other lesions were found in the well nourished cadaver, although specific statements about the condition of the opposite testis, the other endocrine glands, or the secondary sex glands were not made.

* Received for publication, December 10, 1943.

Testicular tumors associated with mammary, prostatic and other changes in 3 cryptorchid dogs were reported by Greulich and Burford.[2] The first dog was a Boston terrier, 4 years of age, which attracted other male dogs like a bitch in heat. The undescended right testis at the caudal pole of the right kidney was occupied by a tumor measuring 48 by 40 by 24 mm., variously diagnosed as a seminoma, an embryoma, and an adenocarcinoma. The right gubernaculum testis extended to the internal inguinal ring. The left testis in the scrotum revealed spermatogenesis arrested at the secondary spermatocyte stage and interstitial fibrosis. The prostate showed metaplasia of the epithelium of ducts and acini to a stratified squamous type. The epithelium in the right ductus deferens was lower than that in the left. Also noted were several "adenomas" in the reticular layer of the adrenal cortex, a possible slight excess of eosinophilic cells in the pars anterior of the hypophysis and some hyperplasia of the ducts and acini of the breasts.

The second dog was a wire-haired fox terrier about 2 years old and sexually attractive to males. At operation a tumor in the right upper quadrant of the abdominal cavity was found to occupy the right testis. It weighed 538.6 gm., measured 125 by 94 by 72 mm., and extended into the left upper quadrant. The histologic structure of this neoplasm was similar to that of the tumor in the first dog. Following operation, the dog lost his sexual attractiveness to other males and died 9 weeks later of generalized peritonitis. Caudal to the right adrenal was an 18 mm. tumor, in structure like the one removed surgically. The left testis in the scrotum showed arrest of spermatogenesis at the spermatogonial stage. The prostate resembled that of a newborn or young dog. The ductus deferentes were intact. Hyperplasia of the cortex and medulla of the right adrenal, some hyperplasia of the ducts and acini of the breasts, and an apparent excess of acidophils in the anterior lobe of the hypophysis were also found.

No estrogenic activity of the tumors in these 2 dogs was demonstrated. The photographs of these 2 animals suggested swelling of the penile sheaths with enlargement of the prepuce as well as relatively conspicuous breasts.

The third dog was a fox terrier, 10 years of age, and bilaterally cryptorchid since birth. For 2 years a swelling had been noticed in the left inguinal region. The histologic structure of the left testicular tumor was similar to that of the neoplasms in the first and second dogs. The right testis in the inguinal canal showed tubules lined by a single layer of spermatogonia and surrounded by fibrous stroma. The prostate was enlarged to three times normal size and disclosed stratified squamous epithelial metaplasia of the ducts and acini. The adrenals

were normal, but the breasts were involved by cystic hyperplasia of the ducts and acini.

Although most pronounced in the third dog, loss of hair and increased pigmentation of the skin over the ventral and ventrolateral surfaces of the abdomen were found in all 3 animals. From a study of the testicular neoplasms in them as reflected in the photomicrographs,[2] the opinion has been formed [3] that these were actually adenocarcinomas, in which the spermatogonia, primary spermatocytes, and probably the sustentacular or Sertoli cells lining the seminiferous tubules undergo proliferation and palisading in an atypical glandular pattern with persistence and stretching of many of the intertubular septa.

A terrier, 7½ years of age, case 1 described by Zuckerman and Groome,[4] had been regarded by his owner as normal until the age of 6 years, except that the animal had "never been known to pursue bitches." For 18 months, the dog had apparently been regarded as a female dog in perpetual heat by the other dogs in the neighborhood. In the last few months increasing drowsiness and enlargement of the nipples had been noted. At necropsy,[4] the testes were stated to be small and in an atrophic condition with spermatogenesis probably in abeyance or much slowed down, although fixation of the tissue was imperfect. In a later communication,[3] it was stated that this same dog had an adenocarcinoma of the testis. Additional autopsy findings [4] included an enlarged prostate with extensive metaplasia to stratified squamous epithelium, a swollen penile sheath and four pairs of enlarged abdominal nipples. In the photograph depicting these last two changes, thinning and loss of hair from the ventral abdominal wall were strongly suggested.

A fox terrier, 12 years old, case 11 of Zuckerman and McKeown,[3] had had for 6 months an enlarged and inflamed scrotum, irregular pigmentation of the skin of the trunk in black patches and increased prominence of the penile sheath and nipples. The swollen right testis showed a large hydrocele and an adenocarcinoma with necrotic metastases in the liver and spleen. The left testis was small. The prostate was enlarged and affected by stratified squamous epithelial metaplasia. Three other dogs, cases 222, 227, 242, described in the same paper, had adenocarcinoma of the testis and stratified squamous epithelial metaplasia of the prostate, although clinical details were not listed. The first dog was a Dalmatian, 15 years of age; the second, a mongrel, was 8 years old; and the third, an Alsatian, was 11 years of age. Another dog, case 225, a fox terrier, 6 years old, was afflicted with an adenocarcinoma of the testis and a carcinoma of the prostate (type not

stated), the two lesions being regarded as occurring together by chance. If the type of carcinoma in the prostate of this dog were actually squamous-celled, the stimulation of the prostate to anaplastic change by the endocrine action of the adenocarcinoma of the testis might be comparable to the formation of adenocarcinoma of the uterine fundus through estrogenic stimulation, as seen in some women with a pre-existing granulosa cell tumor of the ovary. That the presence of adenocarcinoma of the testis in the 15 dogs examined for this lesion by these authors was not associated with any constant changes in the prostate was demonstrated by the histologic diagnosis on the remaining nine prostates, of which four showed great involution of the glandular system; three were normal; and two were much reduced in size.

Experimental Observations from the Literature

The histologic findings in the prostates of 5 puppies (2 normal and 3 castrated) injected with varying amounts of estrone were described by Zuckerman and Groome,[4] who received the tissues from deJongh and Kok.[5] The structures involved included the urethral epithelium, the collecting tubules, the glandular acini and the uterus masculinus. The essential changes consisted of arrest of secretion, epithelial hyperplasia, and epithelial metaplasia. The last two processes graded into each other to result in the conversion of the once single layer of columnar cells into a heavily stratified and desquamating epithelium. The glandular system was transformed into a series of cysts filled with shed epithelial cells and separated from each other by fibrous trabeculae covered on each side by flattened epithelial cells.

Arnold, Hamperl, Holtz, Junkmann and Marx,[6] employing estradiol benzoate and estradiol monobenzoate, described similar changes, including abscesses, in the prostates of 7 adult male dogs, the testes of which showed aspermatogenesis and disappearance of the germinal epithelium.

Huggins and Clark[7] treated 8 male dogs (3 puppies, 3 castrated adults, and 2 adults with cystic hyperplasia of the prostate) with diethylstilbestrol and observed the same changes in the prostate as had Zuckerman and Groome[4] in the material of deJongh and Kok.[5]

Failure of regeneration and loss of hair were reported by Gardner and DeVita[8] in 4 male dogs which received estradiol benzoate beginning in puppyhood. This phenomenon was also observed in 4 adult male dogs fed diethylstilbestrol.[9] The animals described in that communication[9] are the subjects of the following protocols, in which, for the sake of completeness, the data on dosage will be incorporated.

Protocols of Experiments

The first dog, a white and black fox-terrier mongrel, about 1½ years of age, weighing 15½ lbs. and having short hair, received 330 mg. of diethylstilbestrol (stilbestrol) in 20 doses of 5 mg., 19 of 10 mg., and 2 of 20 mg., over a period of 129 days. On the 172nd day, the animal was sacrificed.

The second dog, a white and black cocker-spaniel mongrel, about 3 years old, weighing 18 lbs. and having hair of medium length, received 1790 mg. of stilbestrol in 20 doses of 5 mg. and 169 of 10 mg. in a period of 292 days. Five hundred milligrams of stilbestrol were then implanted subfascially in the right upper quadrant of the ventral abdominal wall. On the 322nd day the animal weighed 17 lbs., was extremely toxic, and was sacrificed.

The third dog, a black water-spaniel mongrel, about 1½ years of age, weighing 17½ lbs. and having long hair, received 1850 mg. of stilbestrol in 185 doses of 10 mg. in a period of 280 days. On the 280th day his weight was 20 lbs., and 500 mg. of stilbestrol were implanted subfascially in the right upper quadrant of the ventral abdominal wall. On the 294th day increased hair loss (Figs. 3 and 5) and depression of libido were prominent symptoms. Further clinical details will be given for this animal, which is still alive and well.

The fourth dog, a black and white collie-scottie mongrel, about 4 years old, weighing about 27 lbs. and having long hair, received 2985 mg. of stilbestrol in 100 doses of 10 mg., 6 of 15 mg., 6 of 20 mg., and 71 of 25 mg. in a period of 291 days. On the 291st day he weighed 24 lbs., and 500 mg. of stilbestrol were implanted subfascially in the right upper quadrant of the ventral abdominal wall. Between the 298th and 305th days, dysuria, hematuria, oliguria, and finally anuria developed. On the 305th day, the animal was sacrificed.

Results

The general thinning of the hair; the loss of hair from the perineum, penile sheath, thighs, buttocks, scapular regions, and circumocular areas (Fig. 1) and from the ventral surfaces of the tail, abdomen, thorax and neck; and the failure of regeneration of hair at areas on the forelegs clipped for venipuncture (Fig. 1) have been described.[9] Libido, which was tested by placing each animal with frequently available female dogs in estrum, was little affected or possibly increased until the 500 mg. dosage level of stilbestrol was exceeded. At the height of medication in the animals receiving more than this amount

of the drug, the libido was definitely greatly depressed, the breasts showed increased prominence, the penile sheath became swollen, the prepuce enlarged and the testes were reduced in size. In the fourth dog the prepuce was superficially ulcerated (Fig. 2).

The tissues from the first, second and fourth dogs were fixed in Zenker's fluid, blocked in paraffin, cut at 6 μ, and stained with hematoxylin and eosin. Those organs usually investigated at a reasonably complete necropsy were examined, but only the alterations observed in the testes, prostate, vasa efferentia, epididymal ducts, ductuli efferentes, penile sheath, breasts, skin, thyroid and adrenals will be described.

Testes. In the first dog the seminiferous tubules were small and spaced relatively far apart. Spermatogenesis in most tubules was arrested at the spermatid stage, although a few small clumps of spermatozoa occupied the lumina of a few tubules. In the second dog the seminiferous tubules were greatly shrunken, the seminal epithelium was developed through the primary spermatocyte stage, and the connective tissue in the stroma was relatively increased. In the fourth dog the seminiferous tubules were very small, most tubules were lined by a layer of spermatogonia, the remaining tubules were replaced by connective tissue and the stroma exhibited increased connective tissue.

Prostate. In the first dog the urethra showed metaplasia to stratified squamous epithelium, the dilated ducts were lined by flattened epithelium, the acinar epithelium was low cuboidal or columnar, and the interacinar stroma was arranged in slender strands. In the second dog stratified squamous epithelial metaplasia involved the urethra, ducts and acini, with sloughing of cornified cells; some masses of necrotic epithelium were seen; and the stroma was diffusely infiltrated by chronic inflammatory cells. In the fourth dog were found extensive epithelial metaplasia of the urethra, ducts and acini to a stratified squamous type; multiple abscesses, and obstruction of the urethra by a conglomerate abscess with hypertrophy of the urinary bladder; subacute cystitis, and chronic pyelonephritis.

Vasa Efferentia, Epididymal Ducts, and Ductuli Efferentes. In the first, second and fourth dogs the epithelial cells lining the vasa efferentia, epididymal ducts, and ductuli efferentes were reduced in height and volume, and frequently showed hyaline transformation of the cytoplasm. The glandular recesses of the ampullae of the vasa efferentia displayed similar changes.

Penile Sheath. In the second and fourth dogs, the epithelial lining of the penile sheath showed intercellular edema, great thickening and hypercornification, characteristics noted [10] in the vaginal epithelium of

female dogs in proestrum. The prepuce of the second dog revealed changes like those to be described for the skin. The epidermis of the prepuce in the fourth dog was largely ulcerated and replaced by proliferating granulation tissue. The epithelium of the surviving epidermis and of the hair follicles showed thickening, intercellular edema and increased granularity of the stratum granulosum. The sebaceous glands were moderately enlarged and scattered sweat glands were dilated and lined by flattened epithelium.

Breasts. In the second dog the epithelial lining cells of the ducts and of a few acini had proliferated and the periductal connective tissue was increased. In the fourth dog the epithelium lining the ducts and acini was hyperplastic, some ducts and acini were dilated and lined by flattened epithelium and the cytoplasm of some acinar cells was hyalinized.

Skin. In the second dog the epidermis was intact. The epithelium of the hair follicles was thinned, the inner follicular cells showed hyaline cytoplasm and the hair follicles were shrunken. Most sebaceous glands revealed disappearance of the lipoid-laden cells, shriveling of the epithelium and shrinkage. Many sweat glands were dilated and lined by flattened epithelium. In the fourth dog the epidermis was intact. Also observed were thinning of the epithelium of the hair follicles, hyaline cytoplasm in the inner follicular cells and shrunken hair follicles. About half the sebaceous glands revealed absent lipoid cells and shriveled epithelium. The other half showed moderate increase in size and well filled lipoid cells. Scattered sweat glands were dilated and lined by flattened epithelium.

Thyroid. In the second and fourth dogs the follicular epithelial cells exhibited shrinkage of the cytoplasm and condensation of the nuclear chromatin. In many follicles, reduced in size, the colloid was scanty or lost.

Adrenals. In the glomerular and fascicular cells of the cortex of the first, second and fourth dogs the cytoplasm was shrunken and the nuclear chromatin was condensed. Cytoplasmic lipid was lost from the fascicular cells as demonstrated by the scharlach R stain on frozen sections of formaldehyde-fixed material.

After the 280th day the third dog received no stilbestrol. On the 396th day he weighed 21 lbs., the hair was almost completely regenerated in the areas from which it had been lost, the swelling of the breasts and penile sheath had subsided and libido had returned as evinced by copulation with female dogs in estrum. On the 583rd day he was generally active, hair was abundant (Figs. 4 and 6), libido persisted and the size of the testes was about normal.

Comment

In my analysis of the data upon 6 dogs with carcinoma of the testis (3 in cryptorchid testes and 3 in scrotal testes) from the literature, the following observations indicating a definite syndrome were evident: (1) adenocarcinoma as the type of neoplasm found and defined by Zuckerman and McKeown; [3] (2) varying degrees of atrophy of the seminiferous epithelium of the opposite testis (one cryptorchid); (3) metaplasia to stratified squamous epithelium in the prostatic urethra, ducts and acini with hypertrophy of the prostate; (4) hyperplasia of the ducts and acini of the breasts with mammary enlargement; (5) swelling of the penile sheath; (6) loss of hair; (7) attraction of other male dogs like a female dog in estrum; and (8) depression of libido. The increased pigmentation of the skin might possibly be included in the syndrome except that patches of pigmentation in the skin of normal dogs are common enough in the experience of investigators who have had occasion to shave the trunks of dogs. The breeds of the 6 animals were Pomeranian, Boston terrier, wire-haired fox terrier (2), fox terrier and terrier. Their ages were "advanced," 4, about 2, 10, 7½ and 12 years.

From the experimental observations in the literature on the effects of estrogens in male dogs and from the findings in the 4 dogs described, the points of similarity to the symptom-complex of feminization associated with carcinoma of the testis become apparent in the following: (1) aspermatogenesis and varying degrees of atrophy of the germinal epithelium of the testes with fibrosis of the stroma; (2) metaplasia to stratified squamous epithelium in the prostatic urethra, ducts and acini with hypertrophy of the prostate; (3) hyperplasia of the ducts and acini of the breasts with mammary enlargement; (4) swelling of the penile sheath; (5) loss of hair; and (6) depression of libido.

In the present experiments the following changes were attributed to the action of the stilbestrol: (1) atrophy of the epithelium lining the vasa efferentia and the glandular recesses of their ampullae, the epididymal ducts and the ductuli efferentes; (2) hyperplasia of the lining epithelium of the penile sheath; (3) partial atrophy of the hair follicles and sebaceous glands of the skin; and (4) partial atrophy of the thyroid gland and adrenal cortex. Evident in the third dog was the recoverability from the hair loss, the depression of libido, the swelling of the breasts and penile sheath and the reduction of the size of the testes.

Summary

A syndrome of feminization in male dogs associated with carcinoma of the testis includes the following features: (1) adenocarcinoma as the type of neoplasm; (2) varying degrees of atrophy of the opposite

testis; (3) stratified squamous epithelial metaplasia of the prostatic urethra, ducts and acini with hypertrophy of the prostate; (4) hyperplasia of the ducts and acini of the breasts with mammary enlargement; (5) swelling of the penile sheath; (6) loss of hair; (7) attraction of other male dogs like a female dog in estrum; and (8) depression of libido. The similarity of the changes produced by both natural and synthetic estrogens to several features of this symptom-complex suggests that the carcinoma of the testis elaborates estrogenic material or at least a substance altered in some metabolic process to a compound having a feminizing action.

REFERENCES

1. Wooldridge, G. H. Carcinoma of the testicle and alopecia in a dog. *Vet. J.*, 1908, **64**, 462–464.
2. Greulich, W. W., and Burford, T. H. Testicular tumors associated with mammary, prostatic, and other changes in cryptorchid dogs. *Am. J. Cancer*, 1936, **28**, 496–511.
3. Zuckerman, S., and McKeown, T. The canine prostate in relation to normal and abnormal testicular changes. *J. Path. & Bact.*, 1938, **46**, 7–19.
4. Zuckerman, S., and Groome, J. R. The aetiology of benign enlargement of the prostate in the dog. *J. Path. & Bact.*, 1937, **44**, 113–124.
5. deJongh, S. E., and Kok, D. J. (Cited by Zuckerman and Groome.[4])
6. Arnold, O., Hamperl, H., Holtz, F., Junkmann, K., and Marx, H. Über die Wirkung des Follikelhormons auf Knochenmark und Blut bei Hunden. *Arch. f. exper. Path. u. Pharmakol.*, 1937, **186**, 1–24.
7. Huggins, C., and Clark, P. J. Quantitative studies of prostatic secretion. II. The effect of castration and of estrogen injection on the normal and on the hyperplastic prostate glands of dogs. *J. Exper. Med.*, 1940, **72**, 747–762.
8. Gardner, W. U., and DeVita, J. Inhibition of hair growth in dogs receiving estrogens. *Yale J. Biol. & Med.*, 1940, **13**, 213–215.
9. Mulligan, R. M. Hair loss in male dogs fed stilbestrol. *Proc. Soc. Exper. Biol. & Med.*, 1943, **54**, 21–22.
10. Mulligan, R. M. Histologic studies on the canine female genital tract. *J. Morphol.*, 1942, **71**, 431–448.

[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 156

FIG. 1. Fourth dog, 232nd day. Hair loss from circumocular areas. Failure of regeneration of hair on forelegs.

FIG. 2. Fourth dog, 305th day. Hair loss from ventral abdominal wall. Swelling of penile sheath and ulcerated prepuce. Prominent breasts at right costal margin in upper left corner and to left of penis.

FIG. 3. Third dog, 294th day, rear view. Hair loss from buttocks, perineum, legs and ventral surface of tail.

FIG. 4. Third dog, 583rd day, rear view. Regeneration of hair on tail in upper part of figure; and on perineum, buttocks, and legs in lower part.

FIG. 5. Third dog, 294th day. Loss of hair from ventral surfaces of thorax and abdominal wall. Prominence of penile sheath and breasts.

FIG. 6. Third dog, 583rd day, close-up of ventral abdominal wall. Regeneration of hair. Hind legs at bottom of figure.

1

2

3

4

5

6

Mulligan

Feminization in Male Dogs

MULTIPLE DIFFUSE FIBROSARCOMA OF BONE *

PAUL E. STEINER, M.D.
(From the Department of Pathology, The University of Chicago, Chicago, Ill.)

In 1936 I encountered a malignant tumor of the skeleton which defied classification. The case was submitted to the Bone Sarcoma Registry of the American College of Surgeons where it stands recorded (no. 2032) as unique. A number of experienced American and European bone pathologists who studied the tumor expressed interesting views, some of which will be given; none had seen a similar tumor. Because it presents several features which are new for bone tumors, it is reported at this time.

Histologically the tumor was a fairly innocent-appearing, long spindle-celled fibrosarcoma of not uncommon appearance. The unusual features consisted of the manner of its growth and of its distribution in the skeleton in association with this histological type. It was widely distributed in bones, which it destroyed. Its disposition was essentially that of the reticulo-endothelial and hemopoietic tissues in the bone marrow of adults. Nowhere did it form any considerable mass or enlargement suggestive of a primary site of origin. There were small metastases in the viscera.

While tumors with this distribution and behavior are not rare, being characteristic of the myelomas, the association of these features with the cytology encountered in this case is unusual.

REPORT OF CASE

L. D., white, male, 43 years old, who was employed as a coal passer on a railroad, was a patient of Dr. L. H. Sloan † at the Illinois Central Hospital, Chicago. Except for dryness and scaling of the skin of the extremities the patient was well until 1934, when he began to have pain in his back. There was no history of trauma. He continued to work until December, 1935, when his back pain became too severe for him to continue, and he entered the hospital on January 16, 1936. The pain was mostly lumbar. It was continuous, did not radiate and was worse at night and on motion.

On the first physical examination the back appeared normal. The left leg was shorter than the right and the pelvis was tilted. The left knee jerk was increased. The left testicle was atrophic. Other findings were normal except for ichthyosis of the skin of the arms and back.

Routine examinations of the blood, urine and stools at this time were negative, as were a gastric analysis and Wassermann and Kahn tests on the blood and spinal fluid.

A lateral roentgenogram of the lumbar vertebra showed no evidence of fracture, dislocation, demineralization, or other bone abnormality. Fluoroscopic examination

* Received for publication, November 23, 1943.

† I am indebted to Dr. Sloan for permission to report this case and to Dr. William Culpepper for the roentgenograms.

of the thorax and its viscera disclosed no abnormality. The esophagus, stomach and duodenum were negative for filling defects when examined after a barium meal, and, except for marked ptosis of the transverse colon, the lower intestinal tract was not remarkable.

On January 28, 1936, a moderate bowing of the spine to the right in the dorsal region and a lordosis in the lumbar region were noted. The left leg was shortened and both femurs were slightly bowed.

The diagnosis at this time was possible beginning Paget's disease.

The patient resumed work on February 1, 1936, but he was unable to continue and he returned to the hospital on May 5, 1936, where he remained until his death on October 30, 1936. He continued to have severe pain in the lower dorsal and lumbar regions. It was not relieved by removal of all of his teeth, traction, or by drugs which later included narcotics. His dorsal and lumbar spine now became fixed, and there was a kyphos in the region of the 8th dorsal vertebra.

On July 28th a neurological examination was negative. Spinal puncture yielded clear fluid with a cell count of 12. A guinea-pig injected with this fluid did not develop tuberculosis. On July 29th new roentgenographic studies showed ". . . . a destructive process involving the bodies of the 4th, 5th, 6th and 9th thoracic vertebrae and the 1st lumbar vertebral body; also the transverse processes; the 12th rib on the left side and the 11th right rib, suggestive of malignancy. Mottled appearance of the pelvis suggestive of atrophic changes is present."

During August two small nodules appeared on the ribs, and in September non-traumatic fractures of the 6th, 7th and 8th ribs were noted. Numbness to the level of the knees developed in September. The urine showed albumin occasionally but was negative for Bence-Jones protein in August and in October. The patient had a severe progressive anemia. The pulse was constantly rapid. During May and June there was a daily afternoon fever with peaks at 99° to 100° F. Early in July a bout of fever with temperatures up to 102° F. was followed by temperatures at the former level. His appetite was poor and he had frequent emeses. Pain was intense. He became dyspneic in October, grew weaker and expired on October 30, 1936. There was no x-ray or radium therapy.

The final clinical impression was that of a malignant tumor metastatic to bone, with the primary site undetermined.

Abstract of Necropsy Examination

The final anatomical diagnoses were: Multiple, diffuse fibrosarcomas of bones; extensive fibrosarcomatous invasion and destruction of the spine with kyphoscoliosis; fibrosarcomatous invasion and destruction of the sternum, ribs, clavicles, pelvis, upper ends of the humeri and femurs, sphenoid and parietal bones; fibrosarcomatous metastases to the lungs, liver, spleen, heart, pancreas, kidneys and adrenals; marked emaciation and anemia; brown atrophy of the heart and liver; generalized serous atrophy of fat; multiple cutaneous nevi; ichthyosis; atrophy of the right kidney and compensatory hypertrophy of the left; slight adenomatous hyperplasia of the adrenals; atrophy of the left testis; traction diverticulum of the esophagus; accessory spleen.

External Appearance. The body was that of an extremely emaciated, deformed and pale white male appearing about 55 years of age rather than the stated 43 years. The estimated weight was 100 lbs. and the

estimated length, 70 inches. The skin showed a marked ichthyosis, being thick, dry, branny and scaling. It was finely cracked and large shreds of dry epidermis could be pulled off almost anywhere. The skin also showed many flat, soft, pale brownish nevi up to 1 cm. in diameter. They were numerous on the trunk, both anteriorly and posteriorly, several dozen being visible. None appeared malignant on gross examination.

The body was deformed. The skull was large. The zygomatic arches were prominent, and the clavicles were very conspicuous. The sternum was deformed, having a deep transverse depression at the level of the junction of manubrium and body and another groove across the lower end of the body. The entire sternum, therefore, lay near the spine, and the chest was flat and long. The costal cartilages were concave and the costochondral junctions were knob-like. Several ribs had small fusiform swellings.

The extremities were wasted. The left leg was about 2 inches shorter than the right. The spine had several abnormal curvatures which will be described with the skeleton.

Body Cavities. Except that the upper two-thirds of the right lung was everywhere adherent to the parietal pleura by fibrous adhesions, the great body cavities appeared normal. The adipose tissue was scanty and dark.

Esophagus. At the level of the tracheal bifurcation there was a diverticulum of the esophagus which admitted half of the distal phalanx of the index finger. Externally it was firmly adherent to a scarred tracheobronchial lymph node.

Parathyroids. The parathyroids appeared about normal in size. They measured: Right upper, 4 by 3 by 1.5 mm.; right lower, 5 by 4 by 2 mm.; left lower, 4 by 4 by 1.5 mm.; left upper, 7 by 4 by 2 mm.

Heart. The heart weighed 300 gm. The epicardial fat was gelatinous and dark, the myocardium was brown and the endocardium was unusually opaque.

Lungs. Each lung had a number of flat, button-like, grayish white, neoplastic pleural nodules up to 1.5 cm. in diameter and not over 2 mm. thick. They had red margins. Scattered throughout the substance of all lobes were numerous hard, grayish white nodules up to 1 cm. in diameter. Similar tumors enclosed and thickened the walls of many bronchi.

Liver. The liver weighed 1510 gm. Through the thin capsule 15 to 18 firm whitish neoplastic nodules up to 2.5 cm. in diameter were visible. The larger tumors had umbilicated centers. Similar tumors were seen throughout the liver substance.

Spleen. The spleen weighed 190 gm. Under the capsule on the diaphragmatic surface there was a nodule, 8 mm. in diameter, and several smaller tumors were visible on the surfaces made by cutting.

Pancreas. One nodule, 4 mm. in diameter, was seen on the anterior surface of the pancreas.

Adrenals. The adrenals were enlarged, together weighing 17.5 gm. One had a yellow nodular area, 2 mm. in size, in the cortex. The other was diffusely enlarged by an infiltration of grayish white tumor, growing in both the cortex and medulla.

Kidneys. The left kidney was markedly atrophic, weighing 31 gm. Its artery was small but not occluded. The right kidney weighed 140 gm. Two firm, whitish nodules of neoplasm, measuring 1.2 and 0.3 cm., were seen in it.

Generative Organs. The left testis was small and fibrotic, while the right appeared normal. The seminal vesicles and the prostate were not remarkable.

Muscular System. The muscles were atrophic and pale. The fat and connective tissue around the muscles was gelatinous, resembling that of serous atrophy.

Skeleton. The calvarium was hard and thinner than usual. An osteolytic tumor, 1.5 cm. in diameter, had eroded both tables in the left parietal bone. The sphenoid was soft and largely replaced by firm, yellowish gray, neoplastic tissue, although its configuration was retained.

The spine showed a number of abnormal curves. In the lower cervical region it turned backward and to the left, forming a kyphoscoliosis, with a sharp angle in the upper dorsal region. It then overcompensated by passing to the right in the lower dorsal region. In the lumbar spine was another kyphoscoliosis to the left. The pelvis was tilted, and it was higher on the left. There were no visible or palpable abnormalities in the bones of the pelvis.

With the knife a wedge-shaped segment was cut from the vertebral column, which appeared comparatively normal externally except for the abnormal curves already described. This wedge of bone was removed with ease from the cervical region to the sacrum. Examination then disclosed that the vertebral bodies were almost entirely replaced by firm, yellowish white tumor. Consequently they were very soft (Fig. 4). Some of the vertebral bodies had collapsed so that they were narrower than the intervertebral disks.

The sternum was angulated and deformed, as previously described. Pieces could be cut from it with ease with the knife at any point (Fig.

4). Both cortex and marrow spaces appeared to be replaced by tumor, although the bone was not enlarged.

The ribs lay near each other because of the partial collapse of the spine and the sinking of the sternum. They could be bent and fractured with ease, and they could be cut with the knife (Fig. 4). The 5th, 6th and 7th left ribs showed slight fusiform enlargements at the sites of previous fractures.

When the lower end of the upper third of the left femur was opened it was found to be of normal hardness. The cortex was thick and the marrow cavity was small. One small neoplastic nodule was seen eroding the cortex from within at this level (Fig. 4).

No bony enlargement resembling a primary tumor site was seen anywhere in the skeleton.

Post-mortem Roentgenograms. Because all bones could not be examined thoroughly at necropsy, post-mortem roentgenograms were made of the trunk, legs and humeri. The femurs showed outward bowing and irregular osteolytic lesions in the center of the upper third, head and neck. Each femur showed a few sharp, osteolytic lesions, about 3 mm. in diameter, in the upper part, but the bones of the leg below this level showed no abnormality. The remainder of the skeleton, except for the distal two-thirds of each clavicle, showed marked demineralization and deformities as described. The cortex of the bones was greatly thinned or entirely absent. Numerous, irregular, confluent osteolytic lesions in each bone were responsible for the osteoporosis.

Histopathology

Only those histopathologic changes which are considered pertinent will be described.

The Tumor. The tumor was composed of spindle cells which, in the sections of bone, were long and slender, but which, in the visceral metastases, were more pleomorphic, although the spindle form was dominant. The nuclei in general were spindle-shaped, but some were irregular. They were usually of medium size but a few giant forms were seen. The chromatin was slightly clumped and scanty, and some hyperchromatic nuclei were found. Mitotic figures were few. The cytoplasm was poorly seen because it was slight in amount and because of the large amount of intercellular fibrillar and mucinous material. The fibrils stained like collagen by the van Gieson, Mallory, and Masson methods. With the Mallory phosphotungstic acid-hematoxylin stain the fibrils were seen to be predominantly adjacent to and not in the cell cytoplasm. Silver impregnation by Perdau's method revealed

much reticulum in the more cellular parts of the tumor. Collagen was abundant in all sections but the greatest amounts were found in the sections of bone. The stroma in many places had a bluish, mucoid appearance, but such regions stained poorly or not at all with mucicarmine.

The cells grew, in general, in the form of broad bundles, but in a few places they had an irregular, whorl-like growth, and elsewhere the growth was even and diffuse. The tumor contained few blood vessels. There was no necrosis. Nowhere did it form bone or cartilage, and no definite osteoid tissue was seen. A fine brown pigment was seen in the tumor in the liver, spleen and bone marrow. This gave a positive Prussian blue reaction for iron and it failed to react positively with Masson's silver impregnation method for melanin. Since a similar pigment was found in these locations apart from the tumor, it was not considered significant.

Bones. The neoplastic growth in many sections of the bones completely replaced the marrow, from which it appeared to have arisen. It destroyed trabeculae and cortex, eroding through the latter in many places. In such places it elevated the periosteum but little or not at all, and it stimulated no periosteal new bone. While occasional tumor cells infiltrated the surrounding soft parts in a few places, no extracortical tumors were formed and the configuration and size of the bones was remarkably well preserved. The tumor was osteolytic, apparently by direct action as well as by stimulation of osteoclasts. Small pieces of necrotic bone enclosed by tumor were found. At one point was seen some calcification in dense collagen.

Vertebral Body. Both cortical and cancellous bone was almost completely destroyed by the tumor, which was here very fibrous. The tumor cells infiltrated the intervertebral disks. Small calcospherites (possibly artifacts) were numerous.

Rib. At this point the rib was almost entirely replaced by tumor, although islands of cortex persisted. There was no sign of repair in an old fracture.

Sternum. Here also was seen total replacement of the bone marrow and nearly complete destruction of the bone itself. Although, as elsewhere, tumor cells penetrated the periosteum, they failed to form extra-osseous tumor masses.

Femur. At the lower end of the upper third of the femur there was a solitary, osteolytic neoplastic nodule, 1 cm. in diameter, eroding the cortex from within. From this point tumor cells were infiltrating the marrow spaces and into the haversian canals. Osteoclasts were

exceptionally numerous here. Elsewhere this bone and its marrow were normal, the latter showing active hemopoiesis.

Visceral Metastases. In the viscera the metastases were fairly sharply circumscribed but infiltrative. The tumor cells tended to invade the surrounding tissues and to incorporate some of the more resistant structures within their limits.

Adrenals. A noncircumscribed metastasis was present in an adrenal. This centered in the medulla and reticular zone, and grew out between the cells of the fascicular zone in delicate linear rows. Another section showed a nodular area composed of atypically arranged cortical cells.

Heart. The endocardial opacity seen grossly was found to be due to an infiltration of the subendocardial region by metastatic tumor, which also extended into the myocardium, particularly along the perivascular connective tissue. A papillary muscle was sheathed by a similar growth.

Lungs. The tumor formed flat masses in the pleura, sheathed some of the blood vessels and bronchi, and infiltrated into the surrounding lung. In some places an exceptionally large amount of mucoid stroma was found.

Liver. In the liver the tumor formed distinct nodules in which the cells showed the greatest degree of pleomorphism and the most whorling.

Spleen. The metastases were poorly circumscribed and highly infiltrative in the spleen.

Kidney. The tumor infiltrated the kidney parenchyma and included the remains of kidney structures. Numerous small metastases, not seen grossly, were present. The stroma was very mucinous, even in the smallest metastases.

Pancreas. One small metastasis was seen in the pancreas.

Miscellaneous. Brief reference is made to additional structures in which no metastases were found.

Parathyroids. No abnormality was seen in the parathyroids except that the oxyphil cells were possibly more numerous than usual at this age.

Skin. There was marked hyperkeratosis and some follicular plugging of the skin. Aside from this the epidermis was very thin, in some places being only six cells thick. The papillae were slender, long, sometimes clubbed and occasionally connected. There was an irregular increase in melanin. The dermis contained a collection of nonpigmented nevus cells. There was no fibrosis or inflammation of the dermis.

Discussion

In summary, this was a purely osteolytic, slightly anaplastic fibrosarcoma which was widely disseminated throughout the hemopoietic and reticulo-endothelial areas of the skeleton, where it replaced the bones without causing enlargement suggestive of a primary site. There were small metastases in many viscera. In addition there was ichthyosis.

Two views about the nature of this tumor present themselves. One is that it represents widespread sarcomatous change in Paget's disease. The other is that it is a peculiar fibrosarcoma probably arising from the medulla of bone and possibly multicentric in origin, and thus related to the myelomas, which it resembles in its distribution and behavior. While it is impossible, from the available evidence, to decide between these two theories, it is my opinion that the latter explanation is more probable. Evidence for both views, and others, will be presented, together with the opinions expressed by some of the consultants.

The main reasons for considering the diagnosis of Paget's disease with sarcomatous change are the pelvic deformity and the bowing of the femurs. The roentgenograms show a change which might be interpreted as new bone formation above the acetabulum, in the trochanters and on the concave side of the femurs.

Against the diagnosis of osteitis deformans is the failure of any section of bone to show the changes characteristic of this disease. There is no new bone formation and the fibrotic tissue can all be regarded as fibrosarcoma. The skull was thin and hard. The tibia showed no roentgenographic changes, and nowhere else are the films typical for this disease. The bones were not thickened or deformed. Furthermore, extensive Paget's disease is unlikely at this age.

Additional reasons for considering this to be a bone sarcoma which arose independently of Paget's disease are its rapid course of 10 months, counting from the time a physician was first consulted. In January roentgenograms were negative and in July destructive lesions were well advanced. At no time were the roentgenographic changes characteristic of Paget's disease.

The question as to whether this tumor was solitary or multicentric in origin cannot be settled at the present time any more than it can with plasma cell or other myelomas. The fact that on the first roentgenographic examination of the skeleton no lesions were found, while 6 months later they were widespread, indicates either simultaneous origin in many places or rapid metastasis. The apparent origin, almost simultaneously, in many bones and the absence of one lesion larger than the others suggest multicentric origin. Multiple, independent

primary tumors in bone, other than myelomas, have been described but they are not common. The distribution in the skeleton, namely, that of the hemopoietic and reticulo-endothelial marrow, cannot be used as evidence either way, because it is common to myelomas and to extensive neoplastic metastasis to bones from within or from without the skeleton.

The presence of marked skin changes, here called ichthyosis, which preceded the development of the bone sarcoma is of interest. No causal relationship has been established between these two conditions, but neither has this possibility been excluded. The thick, dry, scaling skin was stated to have been present for many years, and it became worse as the cancer cachexia developed.

In view of the marked skin changes, as well as some profound alterations in the connective tissues, leading in the connective tissue of bone to sarcoma, a nutritional disturbance, possibly in the nature of vitamin deficiencies, was suspected. No positive evidence for such factors could be found. No history of past dietary deficiencies, indiscretions, or food likes and dislikes could be elicited.

The question also arises as to the relationship, if any, of the nevi in the skin to the osseous sarcoma. Could this be an example of widespread melanosarcoma metastatic into bone, or could it be an extensive disorder of the neuro-ectoderm with nevi in the skin and neurogenic sarcoma in bone? There are good reasons for rejecting the first of these hypotheses: None of the nevi appeared malignant, the cytological appearance of the tumor was not that of melanosarcoma, and there was no melanin in the cells, even by special staining methods. The second idea is less easily disposed of, but neither is there any strong evidence in its favor. While neurogenic sarcomas commonly consist of a spindle-celled growth with whorling, scattered giant cells and a tendency to mucinous degeneration in the stroma, they do not have any known special affinity for the skeleton, either as primary tumors or as metastases. A generalized neurosarcomatosis of the skeleton has not been described to my knowledge, but localized cases have been described. Also, in most areas, the tumor did not resemble the structure of neurogenic sarcoma but was more like that of the ordinary fibrosarcoma. The skeletal changes sometimes seen in cases of von Recklinghausen's neurofibromatosis are unlike those in the present case.

Several consultants, in their reports which are quoted below, emphasized a point which has not hitherto been stressed, namely, that some of the lesions, even in viscera, appeared more like independent foci giving rise to sarcoma than like metastases. This idea is based

on the diffuse, highly infiltrative nature of such areas. There is, however, no gradual gradation from nonneoplastic to sarcomatous connective tissue cells at the margins of such areas but only admixture of these two types of cells. In my opinion the situation is analogous to that found in some carcinomas of the pancreas in which areas are found which appear to consist of precancerous epithelial cells, although probably such is not the case. Grossly, in the present example, the visceral tumors appeared like ordinary metastases. They were distinctly circumscribed.

Excerpts from the written opinions of several consultants are as follows:

Dr. Fred W. Stewart wrote: "I . . . . do not recognize any known disease. Some of the visceral lesions are consistent with metastases but some are not. The fine interstitial fibroses in lung, heart, kidney and adrenal seem part of a general morbid change in connective tissues. The clinical association with extreme ichthyosis is at least remarkable if we merely throw it into the category of Paget's, especially since the bone lesions are not classical of Paget's, nor is extensive Paget's apt to occur at 43."

Dr. E. A. Codman expressed the opinion that: "My best guess is that this is an instance of Paget's disease in which the osteolytic phase far exceeds the osteogenic. . . . The marrow spaces in Paget's disease always show fibrosis. In this instance the fibrosis, by its superlative character, becomes a generalized new growth, just as in many cases of Paget's disease in which a localized sarcoma forms. In this case the process seems to be diffuse. . . . The metastases resemble those in cases of osteogenic sarcoma arising in Paget's disease, *i.e.*, they are nondescript and not very characteristic of having arisen in the bone. I see no evidence to indicate whether the metastases in this case arose from a single tumor or from different foci, but I believe there is evidence of diffuse malignant changes in several bones."

Dr. James Ewing wrote in part: "This case is quite peculiar and I do not remember anything like it. There are some features resembling spindle cell medullary sarcoma of the sclerosing type such as one sees in the medulla of long bones. These features are origin in the medulla, spindle cell structure, osteolytic tendency, absence of bone production and cellular areas suggesting a capacity to give metastases. The multiple tumors may be explained as metastases, but this origin is far from clear. The distribution especially about the spine and in the sternum is very peculiar and suggests rather a primary multiple origin, which is unknown with sclerosing spindle cell bone sarcoma. I do not see enough of the features of Paget's disease to warrant one in including

the case among the sarcomas arising in this disease, where, however, it may possibly belong. The most peculiar and significant feature is the apparent beginning of the proliferative process in organs outside of bone, as adrenal, heart muscle and lung. Here the process is not that of a metastatic lesion, but one finds the very earliest stages of proliferation of spindle cells about fine blood vessels, especially in heart muscle and adrenal. These lesions suggest an universal tendency to proliferation of perithelial cells similar to that seen in some forms of vitamin deficiency. What is the meaning of the extensive ichthyosis of the skin? This has certainly nothing to do with Paget's disease and indicates the presence of some general nutritional dyscrasia."

Dr. Kurt Apitz of the University of Berlin expressed the following opinion and stated that it was that also of Professor Hamperl: "This is—as you rightly put it in your diagnosis—a generalized sarcomatosis of the bones and viscera. The histologic appearance of the tumor is roughly that of a fibrosarcoma, but in its finer details it is quite unusual. I have not seen before such a regular whirl-like arrangement of collagen fibres, except in neurogenic tumors; but the latter origin has little probability for other reasons. I rather think the tumor might be derived from periosteal tissue. If the primary is small, it is quite possible that you cannot differentiate it from the widespread metastatic growth. The sections of the bones are very interesting because they show a peculiar type of calcification, a slight tendency towards osteoid formation, and intensive osteoclastic bone destruction. I do not see how the diagnosis of Paget's disease could be justified. There is only overwhelming metastasis in the marrow, and no other newly formed fibrous tissue, and there is no new bone with mosaic structures."

Summary

An osteolytic fibrosarcoma of bone is described in which the lesions appeared at approximately the same time in many bones, and in which the tumors, although highly infiltrative, retained the normal configuration of the bones. The distribution and extent of the sarcoma was that of the hemopoietic and reticulo-endothelial areas in the skeleton. There were small metastases in many viscera. It is believed that this is an example of a medullary fibrosarcoma somewhat analogous to the myelomas, and that it, like them, might have had a multicentric origin.

[*Illustrations follow*]

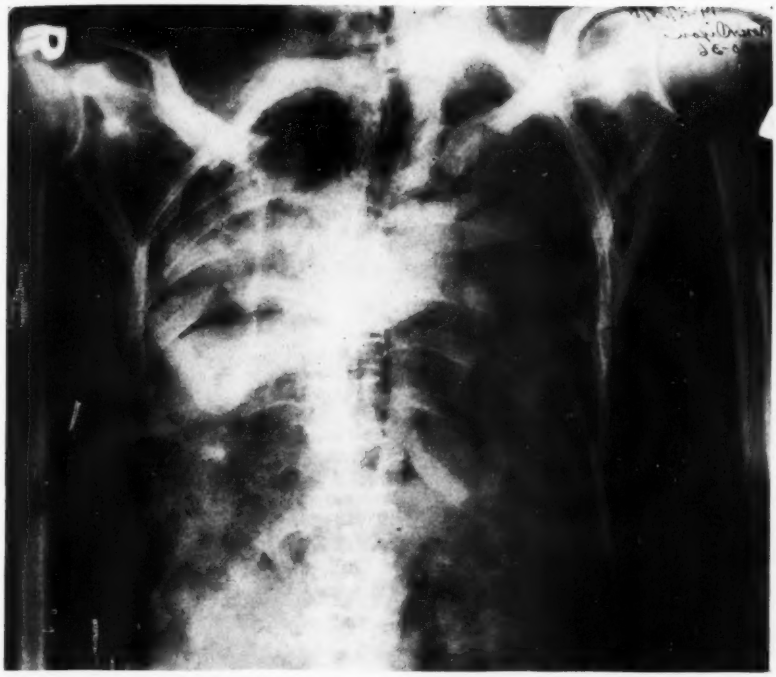
DESCRIPTION OF PLATES

PLATE 157

FIG. 1. Post-mortem roentgenogram showing osteolytic lesions in the humeri, ribs, spine and scapulae. Segments of two upper left ribs were removed and are shown in detail in the lower center of Figure 4.

FIG. 2. Post-mortem roentgenogram showing destructive lesions in the sacrum, pelvis and femora together with slight bowing of the latter. The bone fragment removed from the left femur is shown in Figure 6.

1



2

Steiner Multiple Diffuse Fibrosarcoma of Bone

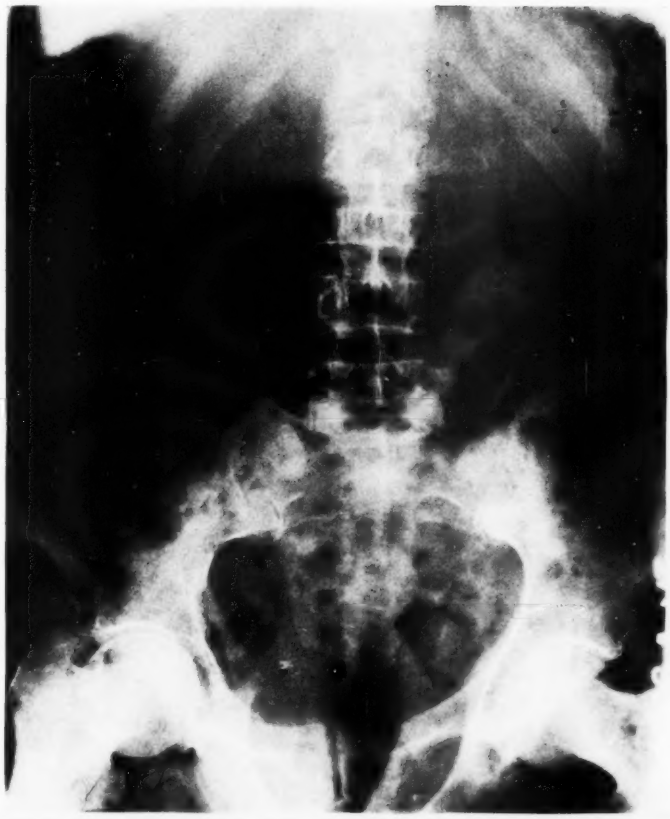
PLATE 158

FIG. 3. Post-mortem roentgenogram of the lower spine and pelvis, showing osteolytic lesions.

FIG. 4. Roentgenogram of a segment of spine showing marked focal and diffuse bone destruction with collapse of several vertebral bodies, and retention of the normal osseous configuration; also (lower left) a segment from the center of the sternum, two left upper ribs (lower center), and a segment of the left femur (lower right), which, by contrast, is underexposed.

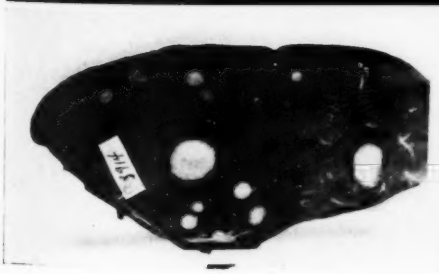
FIG. 5. Portion of liver showing numerous metastases. These were the largest of the visceral metastases.

FIG. 6. Segment of cortex of the left femur showing one osteolytic lesion.

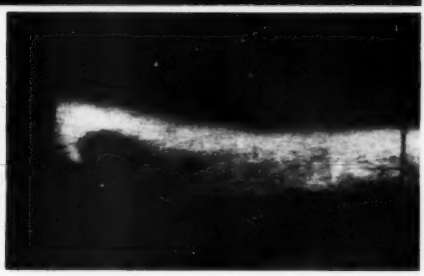


3

4



5



6

Steiner

Multiple Diffuse Fibrosarcoma of Bone

PLATE 159

FIG. 7. Photomicrograph through the center of the sternum showing replacement of the marrow by tumor, partial destruction of trabeculae of bone and invasion, with focal penetration of the cortex and periosteum. × 20.

FIG. 8. Tumor metastasis to the heart. A papillary muscle is enclosed by tumor which also replaces the endocardium and invades the myocardium. × 42.

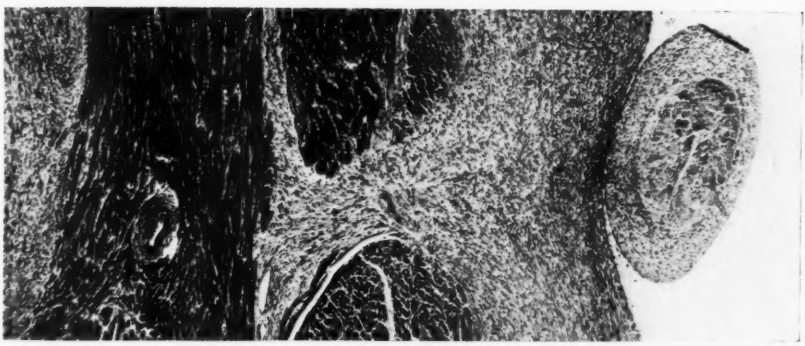
FIG. 9. High power photomicrograph showing the details of the tumor cells in the sternal bone marrow. × 395.

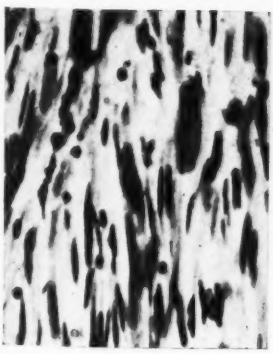
FIG. 10. Lower power photomicrograph showing the most anaplastic and pleomorphic growth which the tumor exhibited. This was in the liver. × 225.

FIG. 11. The manner of tumor infiltration through the adrenal. Similar growth was seen in the pancreas, kidneys and spleen. × 225.

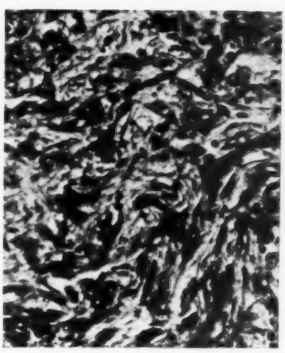
7

8

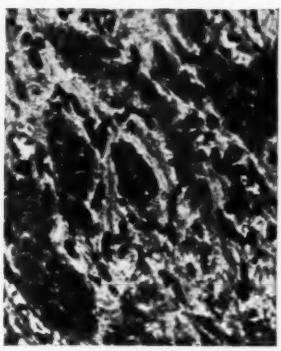




9



10



11

Steiner

Multiple Diffuse Fibrosarcoma of Bone

PRIMARY CARCINOMA OF THE LIVER WITH METASTASES TO BONE

Report of a Case *

Meta A. Neumann

(From the Blackburn Laboratory, St. Elizabeths Hospital, Washington, D.C.)

Primary carcinoma of the liver is a relatively rare neoplasm. A comprehensive survey by Charache [1] gives the incidence of such growths as 0.506 per cent in 509,772 autopsies. These figures were compiled from institutional records in various countries. Other studies [2-4] give a somewhat lower percentage. The ratio is apparently higher among the negroes of South Africa and in Orientals.[5]

In a series of 8,200 autopsies at St. Elizabeths Hospital, primary hepatoma (composed of parenchymal liver cells) has occurred eight times. There were no carcinomas originating in the hepatic bile ducts (cholangiomas). Two cases occurred early in the autopsy series. From these no slides are available for a confirmation of the diagnosis and a histologic description is lacking. Metastatic growths in the lungs were reported in one of them. The six fully verified cases were of the multiple nodular type. The smallest number of tumor nodules was two. In three instances the neoplastic cells showed a more or less uniform structure and little anaplasia. There was an associated cirrhosis in four instances. No other demonstrable primary focus was present.

The following presentation describes the only verified instance of primary carcinoma of the liver with metastases at this hospital. The secondary growths were found only in the osseous system. Charache [1] stated that 20 per cent of all primary carcinomas of the liver metastasize to the lungs; 1.6 per cent to the skeleton. His figures appear representative. In 1937, Bolker, Jacobi and Koven [6] found only nine cases with bone metastases reported in the literature. Three additional occurrences have been reported since their survey.[7-9] A number of the reported cases of primary carcinoma of liver show no extrahepatic metastases.[10-12] The condition occurs much more commonly in males than in females.

Report of Case

History. A colored female, 55 years of age, was admitted to St. Elizabeths Hospital in 1936. As far as is known there were no significant findings in her early history. The patient never went to school, and never learned to read and write. She worked as a domestic and laundress until the day she was hospitalized. The patient had been alcoholic, but in the previous 5 years had refrained from drinking as it interfered with her work.

For some time she had complained of "swimming" in the head, but continued to follow her regular routine until September 13, 1936. On that day she was sent by the police to the House of Detention on the assumption that she was drunk.

* Received for publication, November 30, 1943.

She denied having taken any liquor, but said that she had a headache and a "swimming in her head." The next day she was transferred to Gallinger Hospital, and 2 weeks later she was admitted to this hospital.

She was emaciated, tremulous, and showed beginning arteriosclerotic changes. There was marked asymmetry of the face. Pupils were equal and round, and did not react to light or in convergence. Her tongue protruded tremulously to the right on extension. There was a definite motor speech defect. Marked tremors of face and fingers were noted. The gait was unsteady. There was slight swaying to the right in the Romberg position. Deep reflexes were sluggish. Patellar reflexes were equal but diminished. There was no ankle clonus, and no Babinski sign. There was no evidence of glandular dyscrasia.

The clinical and mental diagnosis, confirmed by serologic and colloidal gold tests, was general paresis.

In the following 5-year period she showed no significant physical changes. She received extensive antiluetic treatment, including several inoculations with tertian and quartan malaria. She was also given an intravenous typhoid vaccine in January, 1937, which was followed by paralysis of the left half of the face, diagnosed as a peripheral paralysis of the seventh nerve. In September, 1937, weakness of the right side was noted by the physician.

In September, 1941, a cyst was observed in the upper portion of her forehead near the midline. The medical protocol stated: "It was crepitant around the margins and was accompanied by two smaller cysts in the neighborhood. X-ray of the skull showed several circular areas of lessened bone density, surrounded by areas of increased bone density." They varied in size from a dime to that of a half-dollar. Blood was withdrawn from these areas on puncture, so no attempt was made to remove tissue for biopsy.

Bones of the pelvis and legs showed no lesions. A tentative diagnosis of multiple meningeal hemangioma was made. Roentgenograms and fluoroscopy of the chest, abdomen and gallbladder were negative.

On December 18, 1941, the patient complained of pain in the left arm. Radiographic examination showed that the entire left humerus was involved in a malignant process. The roentgenologist considered this a metastatic lesion from the skull and modified the original diagnosis to hemangiosarcoma. On December 23, 1941, arteriography of the cranium and contents showed that the large frontoparietal tumor was in the skull and extraneous to the inner table. There was no marked alteration in the normal pattern of the intracranial vessels. Repeated electroencephalograms showed abnormal wave-forms in the right frontal region.

Radiography of the spleen was performed 4 days later. The roentgenologist reported shadows in the regions of both liver and spleen. He stated that "the shadows are not homogeneous, but spotty. They contain areas of radiolucency, which raise a suspicion of absence of organ tissue, with replacement by malignant tissue in these particular spots." On January 20, 1942, a radiograph of the femur showed a small area, about the size of a dime, in the lower third. This was considered as representing another metastatic lesion. During this period the masses in the skull were gradually enlarging. The site of the primary growth was not determined.

The patient grew progressively weaker and died on January 28, 1942.

Necropsy Findings

Autopsy was performed 6 days after death. The body was emaciated. A firm mass was palpated in the midportion of the humerus. There was abnormal movement and crepitation in this region. A large,

rather soft, fluctuant mass, about the size of an orange, was present in the right frontal region. The hair over the tumor was normal. No other abnormalities were noted on external examination.

The scalp was easily dissected from the tumor, and was nowhere invaded. The large frontal tumor was hemispherical in shape, its base measuring about 8 cm. in diameter. It had a smooth, dark red surface, traversed by blood vessels (Fig. 1). Several small hemorrhages were observed. The growth was distinctly fluctuant. It had completely eroded the skull cap, but apparently did not invade the dura. There were four other smaller metastatic lesions in the skull cap; none grossly penetrated the dura. The dura was markedly adherent to the skull cap, and no attempt was made to separate it.

Metastatic lesions were not present in the leptomeninges or brain. There was an old area of infarction in the right third frontal convolution. Cross sections showed that this lesion involved part of the adjacent insula, capsula externa and claustrum.

Examination of the thoracic cage showed no evidence of tumor growth. The heart was small and flabby. Coronary arteries and aorta showed arteriosclerotic changes, and there was evidence of luetic aortitis. The lungs showed congestion, edema, and beginning bronchopneumonia in the left lower lobe.

The liver was markedly enlarged and filled the entire upper half of the abdominal cavity. The surface was nodular and studded with large and small tumor nodules, which varied in size from 0.3 to 10 cm. On palpation the large, dark hemorrhagic nodules were fluctuant, while smaller white tumors were firmer. The largest nodule at the lateral border of the right lobe, just beneath the diaphragm, superficially resembled the cranial growth.

A flat section through the liver gave rise to the impression that there were three types of tumor growth (Fig. 2). The most numerous type produced nodules which were round, and of the yellow color of adrenal cortex. They were extremely soft, even partially liquefied, and showed no structural detail. They seldom averaged more than 1 or 2 cm. in diameter. These growths were usually well circumscribed. The second type consisted of white, firm nodules sharply demarcated from the hepatic parenchyma. The large encapsulated tumor in the right lobe was representative of the third type. It was fairly firm, and its cut surface lacked the homogeneous appearance of the other types. At the periphery there were mottled, hyperemic, yellowish red areas showing numerous hemorrhages. Translucent grayish white bands formed irregular septa between such areas. The central part of the growth was soft and yellowish. It was estimated that more than half of the

liver was replaced by neoplastic tissue. The intact parenchyma was turbid, dark reddish brown, and its architecture was indistinct.

Other parts of the gastrointestinal system were free from disease.

In the genitourinary system there were no significant findings. The kidneys were congested and showed arteriosclerotic changes. There were several characteristic fibromyomata in the uterus. They showed no resemblance to the tumors in liver or cranium.

The lymph nodes showed no trace of metastatic growths. They were nowhere enlarged. The spleen, likewise, showed no abnormal changes.

The right lobe of the thyroid was enlarged to about the size of a small lemon, while the left lobe had normal dimensions. The surface was nodular. No normal thyroid tissue was found on sections. There were many small cysts, which were either empty, filled with grayish, gelatinous material, or with blood-tinged fluid. The solid portions of the gland were grayish and translucent.

The suprarenal glands were of average size and shape. The cortex and medulla were well preserved. In the right adrenal there was a yellowish nodule, 3 mm. in diameter, which was not sharply demarcated from the cortex.

Two metastatic tumors were found in the vertebral column. One had invaded the caudal part of the body of the 7th cervical vertebra. The other growth, in the lower thoracic and upper lumbar regions, had invaded the vertebral disks and adjacent ribs. Both of these tumors were composed of very soft, yellowish tissue, resembling certain of the nodules in the liver. They showed no structural detail.

A 6 inch length of humerus was removed from the middle of the shaft, including the site of neoplastic fracture. The fracture line was roughly parallel to the long axis of the humerus. It was about 4 cm. in length. In the region of the fracture no normal bone cortex was observed. The ill-defined medullary region showed circumscribed, soft, yellow areas and several hemorrhages. There was marked decrease in consistency of the bone, and no sharp distinction between cortex and medulla.

After fixation for several days in formaldehyde solution, the liver and the cranial and other osseous tumors were re-examined. Some of the tumors, which in the fresh state were yellow, had changed to a soft green, resembling patina. This was especially pronounced where the tissue had been in direct contact with formaldehyde. A section through the middle of the humerus did not show this color change. Strangely enough, the green color disappeared after the specimens had been in formaldehyde for about 1 week.

Sections through the large cranial tumor showed dark reddish brown

areas with several small hemorrhages. There were rounded, yellowish areas beneath the thick outer capsule, and adjacent to the dura. The tumor showed central necrosis.

Although the liver seemed the most likely source of the primary growth, the bright yellow color of many of the nodules led to the suspicion that the tumor might have originated in aberrant adrenal tissue. No definite diagnosis could be made from a supravital preparation of a soft, yellowish hepatic tumor, as the faintly granular or vacuolated cells resembled those of either liver or adrenal cortex.

Microscopic Examination

Detailed histologic studies from widely separated areas in the liver clearly established that the primary neoplastic focus was in that organ. The microscopic picture varied considerably in different tumor nodules.

The large encapsulated growth in the right lobe showed marked anaplasia. The normal lobular arrangement was completely lost, but in many areas the tumor cells formed cords separated by narrow clefts. Individual cells, too, were often separated by narrow unstained spaces. The cells comprising the cords were hypertrophied and had deeply stained acidophilic cytoplasm. The darkly stained nuclei frequently assumed bizarre shapes. Lobulated nuclei and signet-ring forms were not uncommon. There were many multinucleated cells; those with two nuclei predominated. The cells showed wide variation in size. Some of them contained bile pigment. Fat vacuoles were numerous.

In general, the clefts between the pseudo-cords were lined with cells which had slender, dark nuclei. Some of the narrow spaces contained a few erythrocytes. The scanty cytoplasm of these cells was occasionally seen to terminate in thin processes.

This growth was highly vascularized. In certain areas the cell cords radiated from an engorged thin-walled vessel (Fig. 3) but never showed a true lobular arrangement. There were many hemorrhages. No tumor thrombi were demonstrable, but occasionally a few tumor cells clung to the lining of a vessel.

There were no bile ducts in the neoplastic tissue. A few isolated ducts were present in the thick connective tissue capsule at the periphery. Dense fibrous tissue separated irregular groups of lobules. A small hemangioma was present in one fibrous band.

Variation in size of cells was a striking feature in all the tumor nodules. In certain areas there was a tendency for the cells to form acini (Fig. 4). Some of the acini were lined by a single layer of cells; in others the lining cells were piled up in disorderly fashion to form several irregular layers. There was no uniformity of structure. Some

of the acini contained red blood cells or a few delicate pink-stained shreds. It should be noted that in those spaces containing erythrocytes there was no endothelial lining; the slender dark cells lining the clefts were on the *outside* of the acini. The irregular anastomosing cords merged gradually with the acini.

Histologically one could not differentiate the white, green and yellow tumor nodules of the gross specimen. Bile pigment was recognized in some areas, and, where hemorrhages occurred, hemosiderin pigmentation. One tumor nodule was composed of an almost solid sheet of epithelial cells. The cells were small and had a pale, foamy cytoplasm.

The hepatic parenchyma which was not involved by the neoplastic process had lost all traces of normal structure. It was the seat of a long-standing cirrhosis. There were isolated cords or small islands of liver cells which showed no true lobular arrangement. Fibrous tissue was moderately increased. There was widespread necrosis. The sinusoids and other venous channels were distended with erythrocytes in varying stages of degeneration. There was extensive hemosiderin pigmentation. Kupffer cells were unusually prominent because of their enclosed pigment. There were a few focal accumulations of lipoid globules. Bile duct proliferation was not marked.

The metastatic growths in the skull, vertebra and humerus showed similar variations in structure. There was no trace of normal bone marrow in the humerus. In the large central neoplasm more or less orderly cords of epithelial cells predominated. They were usually separated by clefts lined with thin, dark nuclei. Many of the spaces between the cells were distended with erythrocytes. The cells were fairly uniform in size in this region but in other secondary growths anaplasia was an outstanding feature. In one tumor from the vertebral column there were many enormous acidophilic cells with lobulated pyknotic nuclei (Fig. 7). These cells merged gradually with alveoli of various sizes, lined usually with smaller cells. The bizarre giant cells sometimes formed acini, but more frequently showed the more characteristic cord structure. In the adipose tissue surrounding the growth there were several medium-sized vessels filled with tumor thrombi (Fig. 8). It was noteworthy that even inside the vessels the tumor cells persisted in reproduction of typical cords separated by narrow spaces which usually contained red blood cells.

In the large tumor of the skull there was extensive necrosis. In the well stained areas many acini had formed (Fig. 6). In other areas the resemblance to hepatic tissue was startling (Fig. 5). The only stroma was the capillary system between the anastomosing cords. The dura showed infiltration with tumor cells in one area. Some of the

metastatic tumor cells contained greenish brown pigment granules. No bile duct formation was found in any of the secondary growths. Fragments of bone were sometimes found deep in the tumor.

The thyroid tumors were classified as fetal adenomas and contained many cysts. The cell picture in no way resembled that of the hepatic or skeletal tumors. The area in one adrenal which aroused suspicion turned out to be only nodular hyperplasia. Many of the organs showed marked congestion and stasis.

Sections from the brain showed very little evidence of an active syphilitic process. The infarct in the frontal lobe was old. There was no evidence of tumor metastases in the central nervous system.

Comment

Most observers agree that the diagnosis of primary liver carcinoma *intra vitam* is difficult and seldom made, and that the duration of symptoms is relatively short, usually only a few months. The symptoms due to lesions in the osseous system, often a spontaneous fracture, may be the first clinical evidence of a physical disturbance. Although the nature of the bony lesion is easily identified by roentgenogram, the location of the primary growth is usually identified only at autopsy.

The most striking feature of these neoplasms is the reproduction of tissue resembling hepatic parenchyma in distant metastases; [5, 6, 13–15] that is, anastomosing cords of epithelial cells separated by endothelium-lined spaces. These cells are apparently able to carry out at least one of the functions of liver cells, namely, the production of bile pigment, as indicated by the green color of the tumors grossly and the histologic identification of the pigment granules. This type of neoplasm is therefore called organoid, rather than histoid, since the metastases give "morphologic evidence of the original physiologic function." [6]

The variegated color scheme of hepatomas has been noted by practically all observers. Mallory [12] commented on the color change from yellow to green in material fixed in formaldehyde, similar to that observed in our case. He stated: "That is the usual reaction when bilirubin is oxidized by formalin. . . . This appearance could mean that the tumor cells were producing bile and that since they were not connected up with any bile ducts it remained in the tumor."

Ewing [15] also discussed the secretion of bile in secondary growths. He stated that: "Bile secretion diminishes with increasing anaplasia." In the reported case it was noted that the tumors which were least typical of liver structure were paler and firmer than the green nodules.

The association of cirrhosis with primary hepatoma is referred to by many authors, and excellently summarized by Ewing.[15] He stated

(p. 722) that "there is little doubt that cirrhosis, and the factors that lead to it, cause degeneration followed by regenerative overgrowth which may become excessive and neoplastic." Charache [1] also felt that the association is too frequent to be a mere coincidence.

There is less agreement on whether the primary focus in the liver is single or multiple. Certain authors [3, 4, 6] believed that those cases which exhibit many tumor nodules throughout the liver show this feature by virtue of intrahepatic dissemination through the portal system. Other observers [1, 15, 16] believed in the multicentric origin of the tumors.

No proof of either theory is furnished by this case. Although samples of tissue were taken from several areas of the liver, all of which were well vascularized, no tumor thrombi were found in any section. In the reported cases which showed only a single hepatic growth it was usually found in the right lobe. Certainly in the case reported here, the right-sided tumor was the largest. It is suggested, however, that the existence of multiple primary foci may be explained as a corollary of the theory that hepatic carcinomas are the sequelae of nodules of regenerating epithelium following cirrhosis. This theory, however, would not offer an explanation for the occurrence of multiple tumor nodules without cirrhosis.

Summary

A case of primary carcinoma of the liver (hepatoma) with metastases to the skeleton is described. The distant metastases reproduced the architecture of the primary, with anastomosing cords of epithelial cells, separated by spaces lined by endothelium. In the areas of better differentiation there was evidence of the formation of bile.

REFERENCES

1. Charache, H. Primary carcinoma of the liver. Report of a case and review of the literature. *Am. J. Surg.*, 1939, **43**, 96–105.
2. Gustafson, E. G. An analysis of 62 cases of primary carcinoma of the liver based on 24,400 necropsies at Bellevue Hospital. *Ann. Int. Med.*, 1937–38, **11**, 889–900.
3. Loesch, J. Primary carcinoma of the liver. *Arch. Path.*, 1939, **28**, 223–235.
4. Gardner, L. W. Primary carcinoma of the liver. *Bull. Harper Hospital*, 1941, **1**, 36–40.
5. Tull, J. C. Primary carcinoma of the liver: A study of 134 cases. *J. Path. & Bact.*, 1932, **35**, 557–562.
6. Bolker, H., Jacobi, M., and Koven, M. T. Primary carcinoma of the liver with bone metastasis. *Ann. Int. Med.*, 1937, **10**, 1212–1221.
7. Hedrick, D. W. Primary carcinoma of the liver with metastases to the bone. Report of a case. *J. Bone & Joint Surg.*, 1937, **19**, 817–820.
8. Cabot case 24302. Hepatoma with metastases to lungs and skeleton. *New England J. Med.*, 1938, **219**, 133–135.

9. Cabot case 27362. Portal cirrhosis of liver; hepatoma with metastases to lungs, bone marrow and adrenal. *New England J. Med.*, 1941, **225**, 386–388.
10. Keeler, C. C. Primary carcinoma of liver. *M. Bull. Vet. Admin.*, 1942, **19**, 143–149.
11. Berns, R. S. Primary carcinoma of the liver. *M. Bull. Vet. Admin.*, 1941, **18**, 211–213.
12. Cabot case 24332. Hepatoma with metastases to lungs, adrenal, lymph nodes and diaphragm. *New England J. Med.*, 1938, **219**, 235–237.
13. Moon, V. H. Primary carcinoma of liver with metastasis to bone. *Arch. Path.*, 1929, **8**, 938–943.
14. Martinez, J. A. Primary carcinoma of the liver. *Lancet*, 1935, **2**, 1293–1294.
15. Ewing, J. Neoplastic Diseases. W. B. Saunders Co., Philadelphia, 1928, ed. 3, pp. 718–731.
16. MacCallum, W. G. A Textbook of Pathology. W. B. Saunders Co., Philadelphia, 1928, ed. 4, p. 1084.

[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 160

FIG. 1. Gross photograph of the cranium, showing metastatic hepatomas.

FIG. 2. Gross photograph of the liver, showing primary carcinoma (hepatoma) and multiple nodular tumors.

1

2

Neumann Primary Carcinoma of the Liver

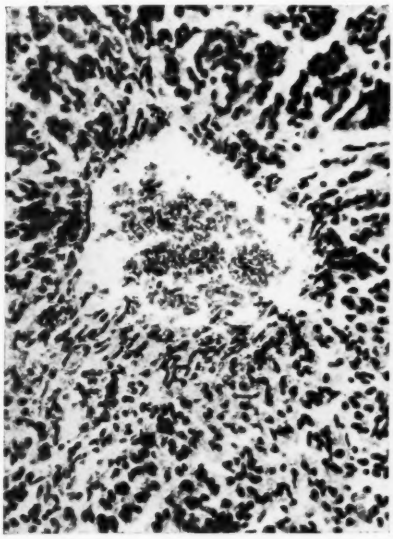
PLATE 161

FIG. 3. Liver. Primary carcinoma. Tumor cells radiating in short cords around thin-walled vessel. Multiplication of slender, dark nuclei around blood space.

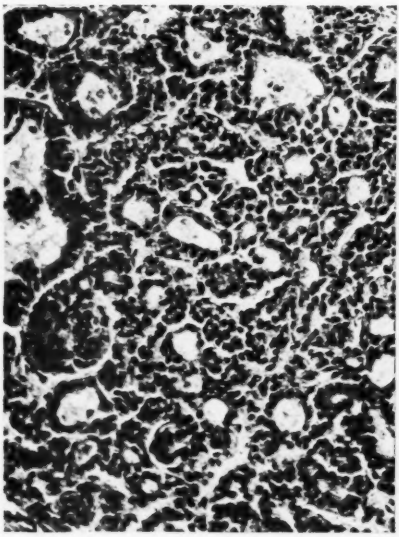
FIG. 4. Tumor cells forming irregular acini.

FIG. 5. Metastatic hepatoma in cranium. Liver-like structure of neoplastic cells.

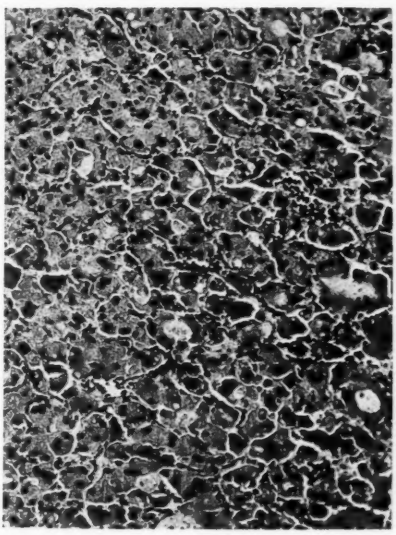
FIG. 6. Metastatic hepatoma in cranium. Tumor cells form branching columns and acini.



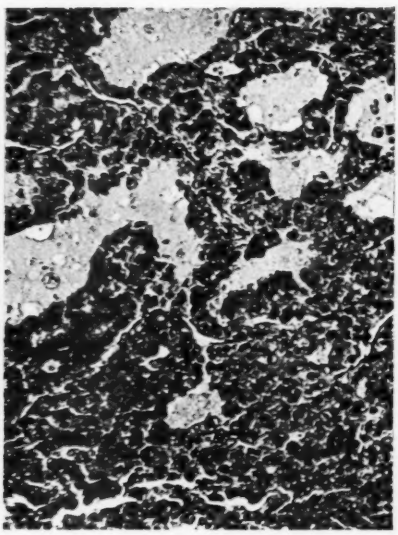
3



4



5



6

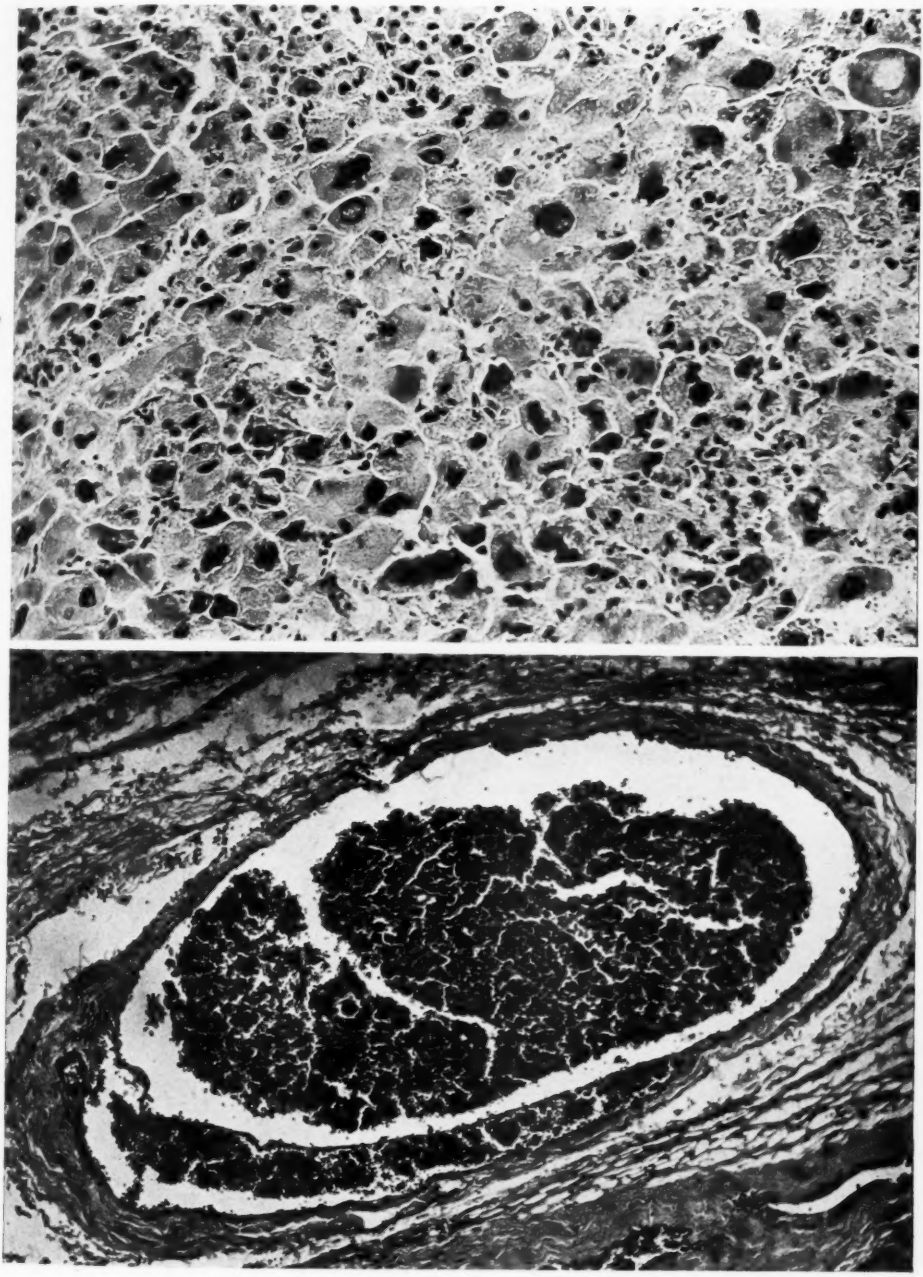
Neumann

Primary Carcinoma of the Liver

PLATE 162

FIG. 7. Metastatic hepatoma in a vertebra. Marked anaplasia of tumor cells. There are slender clefts lined with small, dark nuclei between large neoplastic cells.

FIG. 8. Tumor thrombus adjacent to metastatic growth in vertebral column.



Neumann Primary Carcinoma of the Liver

BRONCHIOLAR ORIGIN OF "ALVEOLAR CELL TUMOR" OF THE LUNG *

PETER A. HERBUT, M.D.
(From the Clinical Laboratories, Jefferson Medical College Hospital, Philadelphia, Pa.)

While in most primary carcinomas of the lung a bronchial origin is easily established, there are occasional cases in which, in spite of a careful search, the point of origin cannot be located. The majority of these, in both the distribution of the tumor and its microscopic appearance, resemble the ordinary bronchogenic variety of carcinoma. A few, however, have a miliary or lobar distribution; infrequently metastasize to distant organs and microscopically are composed of cuboidal or columnar cells lining the alveolar septa. It is this latter group which has been the subject of many previous presentations, under a variety of names, but always with the idea that the primary foci are the septal cells lining the pulmonary alveoli. Recently, Neubuerger and Geever [1] collected from the literature 25 cases of "true alveolar cell tumor" and 15 others which were controversial. Since their publication there have been at least 4 additional cases reported.

Because of the increasing number of these reports,[2-5] it was thought pertinent to draw attention once more to the fact that there is still no convincing evidence that primary pulmonary carcinoma can arise in the alveoli and that all such tumors probably originate in the mucosa of the bronchi and bronchioles. The present report will concern itself with (1) a short summary of the recent views on the status of an alveolar epithelium, (2) the genesis of regenerated alveolar epithelium and (3) a presentation of 6 cases of primary carcinoma of the lung. These were selected from a study of 90 cases which were encountered in the last 6500 necropsies performed at the Jefferson Medical College Hospital. The first 3 to be described meet all the criteria designated as necessary for the diagnosis of an alveolar cell tumor. The remaining 3 cases are typical bronchogenic carcinomas but contain some areas which microscopically are indistinguishable from areas of the former group.

ALVEOLAR EPITHELIUM

For many years the assumption that pulmonary alveoli are lined with a continuous layer of flattened epithelial cells was left unchallenged. Recently, interest in the subject has been revived and at the present time there is much discussion as to the nature of the lining cells. The current views are (1) that there is a continuous layer of epithelial cells (Bensley and Bensley,[6] Miller,[7] Bremer,[8] Cooper [9]); (2) that the lining cells are epithelial but discontinuous (Palmer,[10]

* Received for publication, December 20, 1943.

Macklin,[11, 12] Ham and Baldwin [13]); (3) that the cells are not epithelial but mesenchymal in origin (Rose,[14] Maximow and Bloom,[15] Fried,[16, 17] Barnard and Day,[18] Loosli [19]); and (4) that there are both epithelial and mesenchymal cells present (Sprunt,[20] Ross [21]). Although the problem has been studied from the embryologic, histologic and pathologic standpoints, different observers using the same methods have come to diametrically opposite conclusions. For this reason it would serve no useful purpose to enter into a lengthy discussion of this complex and unsettled problem at the present time. The interested reader may turn to any of the aforementioned authors for a complete review of the various opinions.

Regenerated Alveolar Epithelium

One of the most conspicuous and consistent findings in any chronic inflammatory or fibrotic process of the lung is the presence of cuboidal epithelium forming a continuous lining of the alveolar septa, or spaces in a fibrous tissue stroma. The histogenesis of this epithelium has two interpretations. The older, and by far the more prevalent, view is that the cells arise from former alveolar epithelium which has now resumed its embryonic appearance. This idea has not only been carried down from textbook to textbook (MacCallum,[22] Boyd,[23] Ewing [24]) but has also been reiterated in many recent publications (Wood,[4] Sprunt,[20] Klotz,[25] Neubuerger [26]). Currently it has been discussed at length and rigorously supported by Bell [27] and Geever, Neubuerger and Davis.[28] The second view is that these cells do not originate from the septal cells but that they are outgrowths of the basal cells of the bronchiolar mucous membrane. Although this idea was voiced by Weller [29] in 1929, and described more fully by Fried [16] in 1931, it seems to have been overlooked by most authors.

In an effort to establish the histogenesis of these regenerated cells, a detailed review was made of 60 bronchiectatic lungs together with miscellaneous cases of tuberculosis, organizing pneumonia, interstitial pneumonia and lipoid pneumonia. It is believed that cases of bronchiectasis are particularly adapted for this study because the bronchi and bronchioles are continually irritated by chronic inflammation and therefore have a great opportunity to proliferate. Such was found to be the case in almost every lung examined, and since the findings were so consistent they will be considered here. The two most important and constantly observed changes which have a bearing on the problem at hand were (1) the frequent occurrence of metaplasia of the bronchial mucosa to a transitional or squamous cell type, and (2) the presence of varying amounts of granulation or fibrous tissue which consistently surrounded the involved smaller bronchi and their terminal

ramifications, and the gradual merging of this tissue with the adjoining thickened alveolar septa. Throughout these areas, regular, low cuboidal or even attenuated epithelium lined the alveoli and the spaces within the granulation and fibrous tissue. When enough consecutive sections were made, these lining cells were seen to originate in the mucosa of the bronchioles. Most frequently the regular ciliated bronchiolar epithelium ended abruptly, leaving the basal cells to continue out in a linear arrangement into the adjoining tissue and alveoli (Fig. 1). Less frequently the ciliated mucosa was everywhere intact but focally there were collections of proliferated basal cells. These sent out strands of similar cells which later bifurcated into two limbs, and lined spaces in the surrounding fibrous tissue and, beyond this, the alveoli. In either instance the extensions of basal cells from the bronchioles varied. Occasionally the limbs were short, lined only a small portion of the tissue space or alveolus, and then ended abruptly. More frequently, if several sections were cut from the same block, they could be traced without interruption across several microscopic fields. In all cases the regenerated alveolar epithelium had the same morphologic appearance as did the basal cells of the bronchial and bronchiolar mucosa.

To corroborate, on the other hand, the more prevalent assumption that the regenerated epithelium arises from septal cells, an effort was made to demonstrate a gradual attenuation of the cuboidal cells to form ultimately a blending with the normal inner surface of the alveolar wall, or, to find a transition between the newly formed cells and the phagocytes within the alveolar spaces. Nowhere could such relationships be satisfactorily demonstrated. Furthermore, since preoperatively lipiodol was instilled into all of the lungs, there were frequently seen large macrophages whose cytoplasm was distended with ingested oil (Fig. 2). In many instances these lipophages completely filled the alveoli lined by regenerated cuboidal cells and yet none of the latter showed any evidence of phagocytosis. If regenerated alveolar epithelium arises from septal cells, and since septal cells are known to be phagocytic, one would also expect the regenerated cells to show at least some degree of phagocytosis. Thus it seems that all available evidence substantiates the supposition that the source of the regenerated alveolar epithelium is the basal cell of the bronchiole and not the pre-existing septal cell which has now resumed its embryonic appearance.

Presentation of Cases

Cases Fulfilling the Criteria for Alveolar Cell Tumor

Case 1. A white woman, 50 years old, was admitted to the hospital *in extremis* and died within half an hour. Six months previously she had developed a sudden pain in the chest. This was followed by tightness across the entire thorax, a

hacking, dry cough and later increasing dyspnea. A physical examination was not performed.

At necropsy there were hemorrhagic effusions into the right pleural cavity and the pericardial cavity, and bilateral fibrous and fibrinous pleural adhesions. The entire right lung was gray and diffusely consolidated. Immediately beneath the pleura there were numerous flat, circumscribed or confluent gray nodules measuring 1 to 2 mm. deep and as much as 5 mm. across. Cut surfaces disclosed a homogeneous gray appearance broken only by maplike lines composed apparently of thickened septa. In the medial portion of the upper lobe there was an area of beginning necrosis, 2 cm. in diameter. The left lung contained numerous circumscribed and confluent tumor nodules, measuring as much as 2 cm. in diameter, evenly distributed throughout the parenchyma. A careful examination of the trachea and bronchi disclosed no primary tumor. There were metastases to the mediastinal lymph nodes and liver.

Microscopic sections from each lung were similar. The alveolar septa were slightly thickened because of capillary engorgement or early fibrosis (Fig. 3). Most of the alveoli were partially or completely lined with one to three layers of cuboidal or columnar cells. Although generally these cells were closely adherent to the underlying septa, occasionally there were empty spaces between the lining cells and alveolar walls, while rarely the septa were completely bare and tumor cells were irregularly scattered in the alveolar spaces. The cytoplasm of the tumor cells was always dark-pink-staining and abundant. Occasionally it contained droplets of secretion. The nuclei were either round, oval and of uniform appearance, or showed great variations in shape, size and intensity of staining. Giant cells with 1 to 3 nuclei were abundant. Tumor cells were regularly found within the peribronchial and particularly the perivascular lymphatic vessels and occasionally within the lumina of the bronchioles. The pericardium, mediastinal lymph nodes, liver, adrenals and vertebral bone marrow showed metastases of columnar or cuboidal cells similar to those described in the lungs.

Case 2. A white woman, 48 years old, complained of a persistent cough following pneumonia 1 year previously. She was bedridden the last 9 months, during which time she developed increasing dyspnea and lost 50 pounds in weight. She was emaciated and showed signs of consolidation of the right lung associated with pleural effusion. Roentgenograms of the chest disclosed a homogeneous density on the right side and numerous areas on the left side which resembled "lobular caseous foci."

At necropsy there were fibrous adhesions in the upper part of the right pleural cavity and serous effusions into its inferior portion and into the pericardial cavity. The entire right lung, and particularly its

medial portion, was diffusely consolidated with grayish white neoplastic tissue in which there were small foci of necrosis. The parenchyma of the left lung was infiltrated with innumerable pinhead-sized, gray, firm tumor nodules. The trachea and bronchi showed no primary tumor. There was cancerous involvement of the mediastinal lymph nodes, pericardium and liver.

Microscopic sections of various portions of the lungs showed moderate thickening and fibrosis of the septa (Fig. 4). Tumor cells regularly lined the alveoli either in a linear or papillary arrangement. The cells were mostly tall cuboidal. Their cytoplasm was pink, abundant and nonvacuolated. The nuclei were usually round or oblong and evenly staining, although hyperchromatism was also occasionally present. Giant cells were sparse. At the junction of cancerous and normal lung tissue the septa were thinner, less fibrotic and more congested. Often tumor cells lined only a portion and one side of an alveolus and then ended abruptly. Tumor cells were not found within the lymphatic vessels or the bronchi. Sections of metastatic lesions were similar with the exception that the fibrous tissue strands between the alveoli were broader and denser than those in the lung.

Case 3. A white woman, 52 years old, well until 6 weeks before admission, developed a dry cough, dyspnea, upper abdominal pain, and nausea and vomiting. There were signs of consolidation over the upper portion of the right lung. A roentgenogram of the chest showed haziness on the right side and miliary shadows resembling tuberculosis on both sides.

Necropsy disclosed fibrous and fibrinous pleural adhesions on the left side. The entire left lung was infiltrated with discrete or coalescing gray tumor nodules measuring from 0.7 to 4.0 cm. in diameter. The right lung was essentially similar with the addition that the upper and middle lobes were gray and diffusely consolidated.

Microscopic sections of various portions of each lung showed a moderate thickening of the septa due to capillary engorgement and fibrosis (Fig. 5). While in some fields tumor cells regularly lined the alveoli, most sections showed considerable sloughing of the cells into the alveolar spaces. The cells were tall columnar and contained abundant, dark pink, nonsecreting cytoplasm. The nuclei were round or oval, uniformly stained and peripherally or centrally placed. The most outstanding feature, however, was that even in grossly normal-appearing portions of the lungs there was not a single field that did not contain small, isolated collections of tumor cells. In many areas there were only a few cells in the center of an alveolus, while in others the septa were partially or completely lined in a regular manner. Another outstanding feature was the presence of tumor cells within lymphatic ves-

sels and bronchioles. Sections of metastatic lesions were essentially the same as those in the lungs.

Bronchogenic Carcinomas Containing Areas of Alveolar Cell Arrangement

Case 4. The patient was a man, 69 years old, who complained of cough and expectoration for 14 months and more recently of increasing dyspnea. He showed limited expansion, impaired percussion and decreased breath sounds over the entire right side of the thorax. A roentgenogram of the chest showed homogeneous opacity on the right side obliterating all pneumonic structures.

Necropsy disclosed bilateral serosanguinous pleural effusions. The right visceral pleura was thickened and studded with numerous gray, firm tumor nodules. There was a firm tumor in the right main bronchus causing wrinkling and puckering of the mucosa over a distance of 3 cm. The left lung contained numerous grayish white nodules measuring as much as 6 mm. in diameter. There were metastases to the mediastinal lymph nodes.

Microscopic sections of the primary lesion in the bronchus and various portions of the lungs showed the following four types of tumor cells with gradations from type to type: (1) anaplastic round, spindle or oval cells; (2) masses and sheets of larger cells resembling epidermoid carcinoma without pearl formation; (3) transition from the latter to a glandular type; and (4) cells showing a distinct alveolar arrangement (Fig. 6). In the latter areas the cells were uniformly cuboidal; contained a moderate amount of pink cytoplasm and regularly lined, thickened, and fibrotic alveolar septa. Frequently there were also cells within the alveolar lumina forming structures not unlike renal glomeruli. Throughout the lungs the peribronchial and perivascular lymphatic vessels were conspicuously distended with tumor cells. Sections of metastatic lesions showed the same pleomorphism as did those of the lungs.

Case 5. A white male, 56 years old, complained of pain in the chest for 9 months, dyspnea for 6 months and numbness in the left hand and back for 3 months. There was dullness over the entire right side of the thorax. A roentgenogram of the chest showed an area of diffuse opacity on the right and collapse of the 10th dorsal vertebra.

Necropsy disclosed complete obliteration of the right pleural cavity with dense fibrous adhesions. The right visceral pleura was nodular, gray, and measured as much as 1 cm. in thickness. The lower portion of the main bronchus to the right lower lobe was thickened with gray, firm tissue. Its distal portion was incorporated in a partially necrotic tumor mass which replaced the greater portion of the lobe. The left

lung contained many gray, shot-like tumor nodules, distributed more abundantly in the peripheral portions of the parenchyma. There were metastases to the mediastinal lymph nodes, liver, ribs and lower thoracic vertebrae.

Microscopic sections of the right bronchus and various portions of the lungs disclosed the following distinct types of tumor with transitions from group to group: (1) anaplastic cells with no definite arrangement; (2) larger cells grouped into masses resembling epidermoid carcinoma without keratinization; and (3) regular cuboidal cells lining normal alveolar septa (Fig. 7). The latter contained a moderate amount of pink, occasionally secreting, cytoplasm. The nuclei were round or oval and showed some hyperchromatism. There were occasional giant cells. At the junctions of normal and neoplastic tissue the alveolar septa were often covered only on one side with tumor cells, while the other side was entirely bare. Frequently, only a part of an alveolus was lined and at this point the cells terminated abruptly. Sections of metastatic foci were composed of anaplastic or stratified squamous epithelium.

Case 6. A white man, 57 years old, complained of pain in his bones and a nonproductive cough for 4 months. Breath sounds were increased over the apices of both upper lobes. A roentgenogram of the chest showed areas of increased density at the apices of the lungs, interpreted as tuberculosis.

Necropsy disclosed bilateral apical pleural adhesions and tuberculosis of the underlying lung parenchyma. In addition the upper half of the right upper lobe was almost completely replaced with a grayish white, firm tumor which blended gradually with the adjoining parenchyma. The main bronchus to this lobe was normal but its branches were definitely stenotic as they entered the neoplastic tissue. There were two tumor nodules in the lateral portion of the left lung, and metastases to the lymph nodes, adrenals, liver, bone marrow and kidneys.

Microscopic sections of various portions of the right upper lobe disclosed areas of squamous, cuboidal, or columnar epithelium. Frequently the same field showed two types of cells (Fig. 8). Often one alveolus contained a mass of squamous cells and the adjoining surface of the septum was bare. The opposite surface of the same septum was covered with a single or double row of tall cuboidal or columnar cells. The cytoplasm was deep pink and frequently vacuolated. Many of the nuclei were round or oblong, evenly stained and basilar in position. Others varied markedly in shape and size, showed considerable hyperchromatism and were irregularly arranged. Giant cells were frequent.

The alveolar spaces contained varied amounts of pink or blue mucoid material. Sections of metastatic lesions showed a similar pleomorphism of cells.

Discussion

Since the characteristics of "alveolar cell tumors" are so typically epithelial, most authors have called them carcinomas. Their origin from septal cells is the most obvious explanation, and would be entirely acceptable, were it not for the fact that the genesis of septal cells is so much in doubt. If they were definitely shown to be epithelial, there would be no further controversy, but, since the view that they are mesenchymal is gaining more and more advocates,[1] it is extremely difficult to reconcile the supposition that a mesenchymal cell can give rise to a typical carcinoma. To say simply that the germ cells are not specific and that it is possible for a mesenchymal cell to give rise to tumors with epithelium-like characteristics seems hardly enough. Until, however, the origin of septal cells is settled it is rather purposeless to argue the pros and cons of the genesis of alveolar cell tumor on this basis alone. One must, therefore, turn to other available evidence. This is offered in a consideration of the genesis of regenerated alveolar epithelium and in a study of the pleomorphism of the much more commonly observed bronchogenic carcinoma.

Those authors who believe in an alveolar cell tumor as a separate entity invariably point to the frequency with which regenerated alveolar epithelium is found in a variety of chronic inflammatory and fibrotic processes of the lungs. They state further that these regenerated cells arise from septal cells and that sometimes regeneration proceeds so far that the process might even be considered as cancerous. While it is agreed that alveolar cell tumor and regenerated alveolar epithelium have a common origin, and, in fact, that the former may even be preceded in some instances by the latter, I have failed to find any convincing evidence that the septal cell is the parent cell. On the other hand, the focal proliferations of the basal cells of the bronchi and bronchioles with their extensions into the surrounding tissue; their direct linear continuations with the regenerated alveolar cells; the identical morphologic appearance of the two cells; the failure to demonstrate satisfactory transitions between the regenerated cells and the alveolar phagocytes, on the one hand, and the normal inner surface of the septa, on the other; and finally, the absence of phagocytosis by the regenerated epithelium, all point to an origin from the basal cell of the bronchiolar mucosa. I cannot agree with Geever, Neubuerger and Davis [28] when, referring to the downgrowth of bron-

chogenic epithelium, they say that "the cells lining such spaces were high-columnar . . . ." On the contrary, in the cases studied here, the cells were invariably low cuboidal and in many instances were even somewhat attenuated. This is well illustrated in the accompanying photomicrograph (Fig. 1). Furthermore, while in this report special reference is made to cases of bronchiectasis, the regenerated cells differed in no way morphologically from the regenerated cells observed in the other pulmonary infections reviewed for this communication.

The polymorphism of bronchogenic carcinoma is well known and has been adequately considered by Fried,[16] Klotz,[25] Weller,[29, 30] Boyd,[31] and others. The instances of bonified bronchogenic carcinoma recorded in this presentation illustrate several points of interest. In cases 4 and 5 the primary sites were definitely established, and, while in each, many cells were anaplastic and others squamous in formation, there were some areas which were typically alveolar in distribution. This was particularly true of case 5 in which the cells were not only tall cuboidal, and so resembled those in cases 1 and 3, but at the junction of normal and neoplastic tissue they lined the alveolar septa in the same manner as did those in case 2. In each of these, one side of a septum was often covered with tumor cells while the opposite side of the same septum was bare. Frequently, too, the cells lined only the tumor portion of a septum and then ended abruptly as though they did not have time to cover the entire surface. Case 4 was included in this report because, while the cells did not resemble those in the first three cases, there are nevertheless recorded instances of alveolar cell tumor in man in which the cells are somewhat lower and in which they are often so arranged as to resemble renal glomeruli. Finally, case 6 illustrates (1) that a primary focus is not always located definitely even in an otherwise typical bronchogenic carcinoma (although the bronchi to the upper lobe were unquestionably stenotic), and (2) the close association of different varieties of cells in the same tumor and even in the same microscopic fields. More specifically, it showed alveoli filled with squamous cells and bare adjoining septa, while the opposite sides of the same septa were lined, not with squamous cells, but with cuboidal cells of exactly the same appearance as those found in a typical alveolar cell tumor.

One of the chief objections to the supposition that alveolar cell tumors arise in the bronchioles has been the failure to locate the site of origin. This, however, is not surprising for, by the time the tumor has reached a size to cause death, such a primary focus will have long been overgrown by neoplastic tissue, or, at least so altered that an

origin from a bronchiole could not be ascertained. To be sure, in case 1 there were several bronchioles whose entire walls were infiltrated with neoplastic tissue yet no one could say positively whether the cancer arose within the bronchiole or infiltrated from without. The answer to this question will lie solely in the examination of very early tumors found incidentally at necropsy. The literature contains only one report, that of Ravenna [32] in 1909, in which the neoplasm might have been called an alveolar cell tumor had the author not found a gradual transition from normal bronchial epithelium to cancerous tissue, thus establishing a bronchiolar origin.

Although most authors who believe in the existence of an alveolar cell tumor as a separate entity also believe that there are multiple foci of origin, my experience with the cases reviewed for this report makes such a supposition entirely unnecessary. To begin with, it is generally true that carcinomas arise from a single focus and that the remaining growths are secondary. It is also true that often the primary site is extremely small and metastatic lesions so disproportionately large that they first attract attention. If the primary focus in the lung is in the periphery, it may easily be overshadowed by secondary tumors. Since bronchogenic carcinomas ordinarily are considered to arise from one bronchus and the multiple growths throughout the rest of the lungs are considered to be metastatic, why should the same explanation not suffice for carcinomas with an alveolar distribution? In cases 1 and 3, which showed such a distribution of neoplastic cells, many sections disclosed neoplastic tissue within the lymphatic vessels and bronchioles. It is a simple matter for the cells to break through the thin lymphatic walls, or to move peripherally along the lumina of the bronchioles until the air sacs are reached. Such a spread, and particularly by way of the bronchial and bronchiolar lumen, is not limited to tumors with an alveolar distribution, for, in this series of 90 cases, there were many instances in which alveolar spaces contained nests of squamous or anaplastic cells. In fact, in 2 cases which I have recently examined at autopsy, a solid pneumonic spread first attracted attention and was considered as organizing pneumonia, but it was found that a bronchogenic carcinoma was the source of the pneumonic process. Microscopically the alveoli were filled with anaplastic spindle cells but none lined the septa. Apparently the arrangement of neoplastic tissue in the alveoli is dictated by the type of cell present. If the basal cell of the bronchus or bronchiole gives rise to squamous or anaplastic cells, the distribution of these cells will be that of an exudate in acute pneumonia, that is, as clumps in the alveolar spaces and not lining the septa. If, on

the other hand, the basal cell gives rise to columnar or cuboidal cells, then, following the inherent characteristics of these cells to line spaces, they will be regularly distributed along the septa forming the well known alveolar arrangement.

Summary and Conclusions

1. A review of the current literature regarding the status of an alveolar lining of the lungs indicates much diversity of opinion. Therefore the possibility of cancer developing from septal cells cannot be either confirmed or denied on this basis alone.

2. Considerable evidence is presented to show that regenerated alveolar epithelium arises not from septal cells but from the basal cells of the bronchioles.

3. On this basis alveolar cell tumors are also considered to arise from the basal cells of the bronchioles and not from septal cells.

4. This is given additional support from a study of the pleomorphism of the more commonly observed bronchogenic carcinoma in which squamous or anaplastic cells and cuboidal or columnar cells are found side by side in the same tumor and even in the same microscopic field.

5. It is believed that the parent cell in all cases of primary carcinoma of the lung is the basal cell of the bronchial or bronchiolar mucosa. The distribution of the subsequent tumor is dependent upon the further differentiation of the cells. If they are anaplastic or squamous, they will be either irregularly distributed throughout the lungs or will occupy the alveolar spaces just as does an inflammatory exudate in pneumonia. If they are cuboidal or columnar, they will regularly line the septa producing the well known alveolar arrangement. While only a few tumors are indisputably of one group or the other, there are many intermediary transitions between the two.

REFERENCES

1. Neubuerger, K. T., and Geever, E. F. Alveolar cell tumor of the human lung. *Arch. Path.*, 1942, **33**, 551–569.

2. Desmeules, R., Rousseau, L., Giroux, M., and Sirois, A. Amiantose et cancers pulmonaires. *Laval méd.*, 1941, **6**, 97–108.

3. Dacie, J. V., and Hoyle, C. Malignant adenomatosis (alveolar cell tumour) of the lung. *Brit. J. Tuberc.*, 1942, **36**, 158–165.

4. Wood, E. H., Jr. Unusual case of carcinoma of both lungs associated with lipoid pneumonia. *Radiology*, 1943, **40**, 193–195.

5. Sims, J. L. Multiple bilateral pulmonary adenomatosis in man. *Arch. Int. Med.*, 1943, **71**, 403–409.

6. Bensley, R. D., and Bensley, S. H. Studies of the lining of the pulmonary alveolus of normal lungs of adult animals. *Anat. Rec.*, 1935–36, **64**, 41–49.

7. Miller, W. S. The Lung. C. C. Thomas, Springfield, Ill., 1937, p. 56.

8. Bremer, J. L. Pulmonic alveolar epithelium. A round table conference. Edited by Macklin.[11]

9. Cooper, E. R. A. A histologic investigation of the development and structure of the human lung. *J. Path. & Bact.*, 1938, **47**, 105–114.

10. Palmer, D. M. Pulmonic alveolar epithelium. A round table conference. Edited by Macklin.[11]

11. Macklin, C. C. Pulmonic alveolar epithelium. A round table conference. *J. Thoracic Surg.*, 1936–37, **6**, 82–88.

12. Macklin, C. C. The silver lineation on the surface of the pulmonic alveolar walls of the mature cat, produced by applying weak silver nitrate solution and exposing to sunrays or photographic developer. *J. Thoracic Surg.*, 1938, **7**, 536–551.

13. Ham, A. W., and Baldwin, K. W. A histological study of the development of the lung with particular reference to the nature of alveoli. *Anat. Rec.*, 1941, **81**, 363–379.

14. Rose, S. B. The finer structure of the lung, with special reference to its vascular character and its pathologic significance. *Arch. Path.*, 1928, **6**, 36–47.

15. Maximow, A., and Bloom, W. A. A Textbook of Histology. W. B. Saunders Co., Philadelphia, 1930, p. 450.

16. Fried, B. M. Primary carcinoma of the lung. Bronchiogenic cancer—a clinical and pathological study. *Medicine*, 1931, **10**, 373–508.

17. Fried, B. M. Pulmonic alveolar epithelium. A round table conference. Edited by Macklin.[11]

18. Barnard, W. G., and Day, T. D. The development of the terminal air passages of the human lung. *J. Path. & Bact.*, 1937, **45**, 67–73.

19. Loosli, C. G. The structure of the respiratory portion of the mammalian lung with notes on the lining of the frog lung. *Am. J. Anat.*, 1937–38, **62**, 375–425.

20. Sprunt, D. H. Pulmonic alveolar epithelium. A round table conference. Edited by Macklin.[11]

21. Ross, I. S. Pulmonary epithelium and proliferative reactions in the lungs. A study of the cellular response in lungs after intratrachial injection of toxic and nontoxic foreign substances. *Arch. Path.*, 1939, **27**, 478–496.

22. MacCallum, W. G. A Textbook of Pathology. W. B. Saunders Co., Philadelphia, 1924, ed. 3, pp. 597, 667, 744.

23. Boyd, W. A Textbook of Pathology. Lea & Febiger, Philadelphia, 1938, ed. 3, p. 470.

24. Ewing, J. Neoplastic Diseases. W. B. Saunders Co., 1940, ed. 4, p. 879.

25. Klotz, O. An address on cancer of the lung; with a report upon twenty-four cases. *Canad. M. A. J.*, 1927, **17**, 989–996.

26. Neubuerger, K. Primary multiple alveolar cell tumor of the human lung. Report of a case. *J. Thoracic Surg.*, 1941, **10**, 557–565.

27. Bell, E. T. Hyperplasia of the pulmonary alveolar epithelium in disease. *Am. J. Path.*, 1943, **19**, 901–911.

28. Geever, E. F., Neubuerger, K. T., and Davis, C. L. The pulmonary alveolar lining under various pathologic conditions in man and animals. *Am. J. Path.*, 1943, **19**, 913–937.

29. Weller, C. V. The pathology of primary carcinoma of the lung. *Arch. Path.*, 1929, **7**, 478–519.

30. Weller, C. V. Entdifferentiation in primary carcinoma of the bronchi and lungs. *J. Cancer Research*, 1929, **13**, 218–238.

31. Boyd, W. Notes on the pathology of primary carcinoma of the lungs. *Canad. M. A. J.*, 1930, **23**, 210–217.

32. Ravenna, H. Contribution a l'étude du cancer primitif du poumon. *Arch. de méd. expér. et d'anat. path.*, 1909, **21**, 87–101.

[*Illustrations follow*]

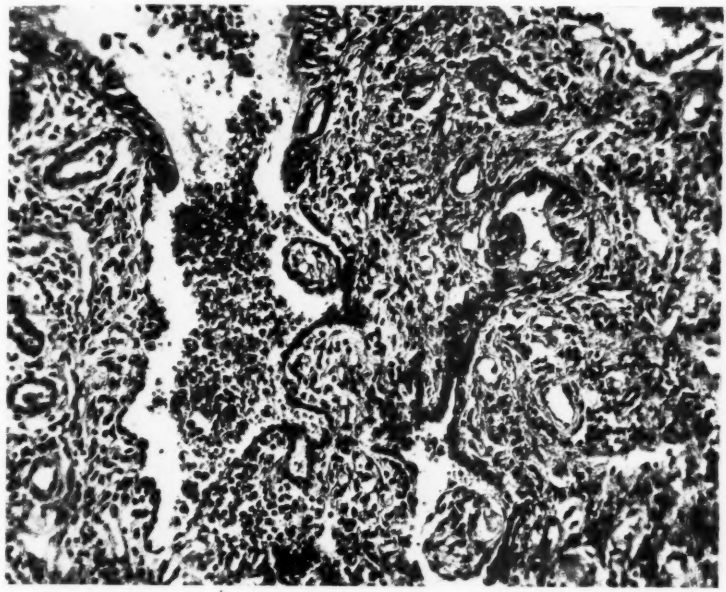
DESCRIPTION OF PLATES

PLATE 163

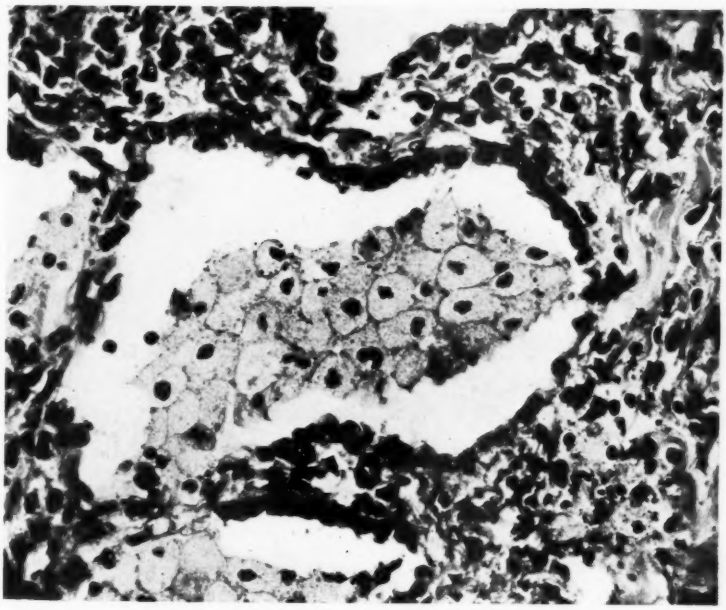
FIG. 1. Section of a bronchiectatic lung showing two bronchioles lined with ciliated columnar epithelium. The larger one, at the left, shows an abrupt termination of the ciliated epithelium and a continuation of the basal cells into the surrounding fibrous tissue. The lumen contains débris and macrophages. The smaller bronchiole, at the right, shows a focal proliferation of basal cells with a cord-like extension into the surrounding tissue and beyond this a bifurcation into two limbs which line a tissue space. × 200.

FIG. 2. Section of a bronchiectatic lung showing regenerated alveolar cells lining an alveolus. The lumen contains many lipophages. There is no phagocytosis by the regenerated alveolar cells. × 400.

1



2



Herbut Bronchiolar Origin of "Alveolar Cell Tûmor"

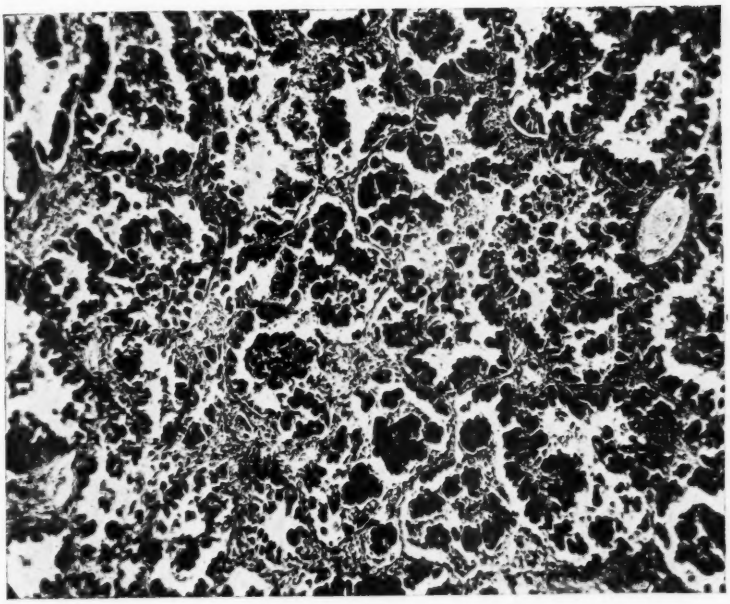
PLATE 164

FIG. 3. Case 1. Section of a lung showing tall cuboidal or columnar cells regularly lining the alveoli. There is some sloughing of the tumor cells into the alveolar spaces. × 100.

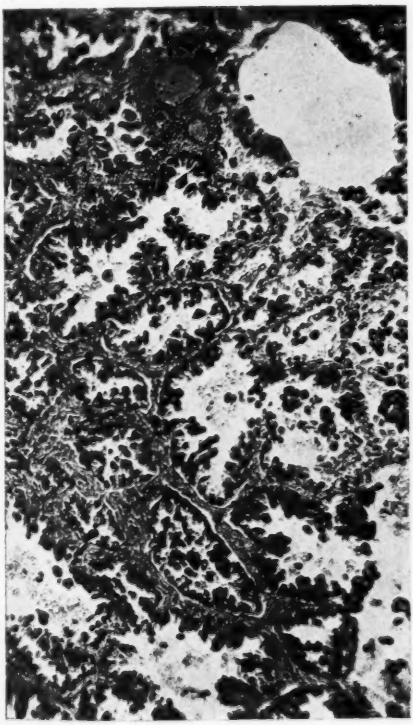
FIG. 4. Case 2. Section of a lung showing considerable fibrosis of the septa lined with tall cuboidal tumor cells. In the transition from tumor to normal lung the tumor cells cover only a portion of a septum. × 100.

FIG. 5. Case 3. Section of a lung showing thickened alveoli lined with tall columnar cells. There is considerable sloughing of the cells into the alveolar spaces. × 100.

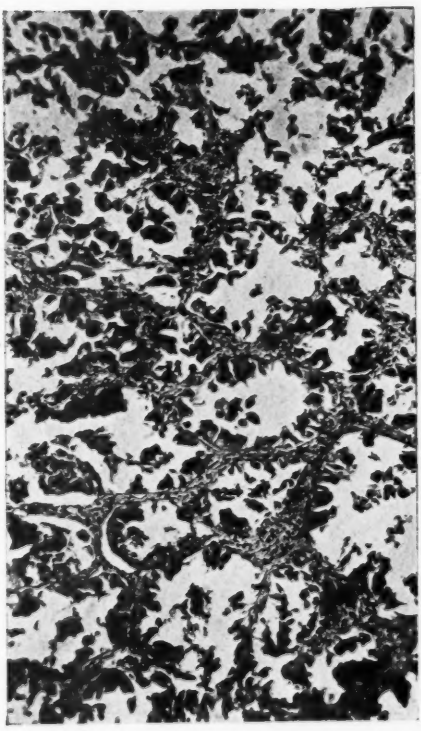
3



4



5



Herbut

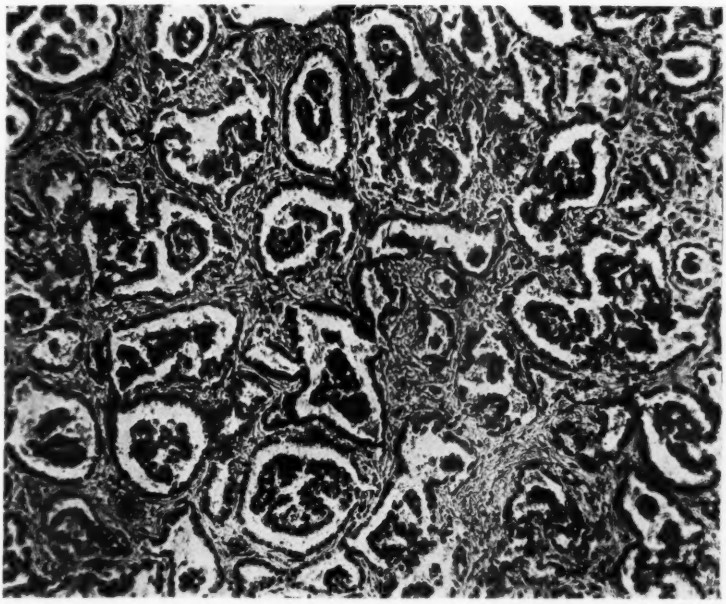
Bronchiolar Origin of "Alveolar Cell Tumor"

PLATE 165

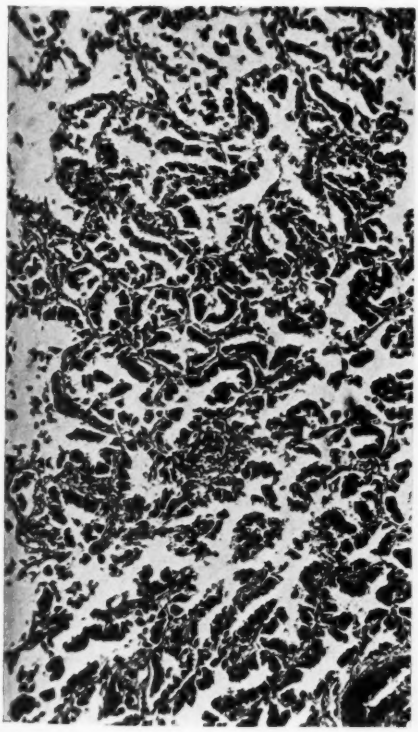
FIG. 6. Case 4. Section of a lung showing greatly thickened alveolar septa lined with cuboidal cells. There are numerous collections of tumor cells in the lumina, producing structures resembling renal glomeruli. × 100.

FIG. 7. Case 5. Section of a lung showing tall cuboidal cells lining relatively normal alveolar septa. In the transition from neoplastic to normal tissue in the upper left corner the tumor cells often line only one side of a septum. × 100.

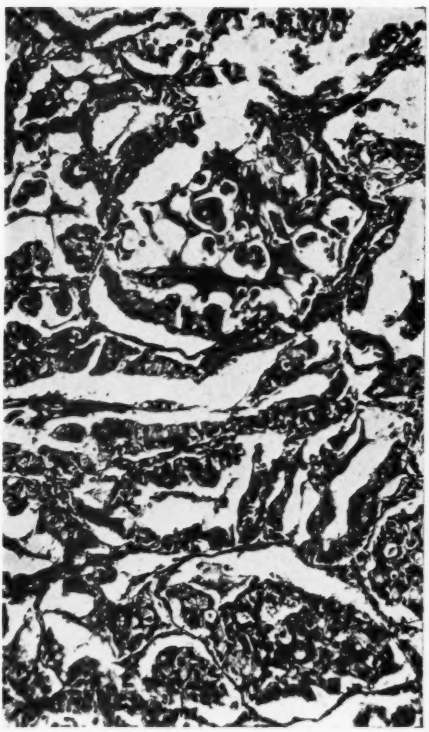
FIG. 8. Case 6. Section of a lung showing nests of squamous cells filling the alveoli below and columnar cells lining the alveolar septa above. In the transition between the two the same septum in adjoining alveoli is lined on one side with columnar cells while the other side is bare and the lumen is filled with squamous cells. × 100.



6



7



8

Herbut

Bronchiolar Origin of "Alveolar Cell Tumor"

PROLIFERATIVE ACTIVITY IN BRUNNER'S GLANDS *

PHILIP H. HARTZ, M.D., and ARY VAN DER SAR, M.D.
(From the Public Health Service, Curaçao, N. W. I.)

In a study on Brunner's glands of the duodenum during which more than 1000 duodena were microscopically examined, Robertson [1] came to the conclusion that: "Hardly any more stable and colorless set of tissues can be found in the body. . . . In the presence of acute inflammatory conditions in the mucosa of the duodenum, even with erosions or penetrating ulcers, Brunner's glands are apparently noncombatants. . . . The glands become *spurlos versenkt.*" Only *twice* could evidences of proliferative activity be observed in the region of duodenal ulcers. "These consisted of hyperchromatism of the nuclei, decrease in the cytoplasm and occasional mitosis."

In view of the reactivity shown by other tissues and glands, possessing a higher degree of morphological differentiation, and of Bloom's [2] statement that in pathological conditions, for instance after local injuries, almost every type of epithelium in the human organism may display a considerable ability for regenerative proliferation, this absence of proliferative activity seemed improbable and we therefore began examining the duodena of our routine autopsy material for the signs of proliferation in Brunner's glands, *i.e.,* mitotic divisions. From the beginning we gained the impression that Brunner's glands are not so dull as Robertson [1] contends and that interesting variations in the cytological details of the glandular epithelium and in the numbers of enterochromaffin cells and oxyphilic cells present in the glands could be observed. After examining a short series of cases our results concerning the proliferative activity of the glands were so different from those of Robertson that we decided to report them.

MATERIAL AND METHODS

During several months the duodenum from every autopsy, which could be performed 1 hour or less after death, was examined. In this way 22 duodena were collected and to these 22 cases 2 earlier examples, in which acute ulcers had been found, were added. The ages of these 24 subjects ranged from 15 months to 71 years. They were all colored people.

Several times the duodenum was removed and fixed immediately after the opening of the abdomen. In most cases Orth's fluid was used for fixation. This fluid is an excellent fixative for the cytological details of the cells of Brunner's glands and for the enterochromaffin cells, the

* Received for publication, November 23, 1943.

granules of which are stained bright yellow, so that they are easily distinguished without special staining methods. Helly's fluid has the same advantages, but paraffin embedding after this fixative causes much shrinking of the tissues.

Péterfi's methylbenzoate-celloidin method for paraffin embedding[3] was used and the sections were stained with hematoxylin and azophloxine and orange-eosin-toluidine blue. Only that part of the gland which was lying below the muscularis mucosae was considered and the collecting ducts of the lobuli excluded as much as possible.

Reports of Cases

Case 1

The patient was a colored girl, 5 years old, upon whom autopsy was performed 40 minutes after death. Anatomical diagnoses: meningitis tuberculosa; duodenitis erosiva; melaena.

Microscopical Examination of the Duodenum. In several places there was extensive ulceration which reached to and sometimes into the submucosa. The mucosa between the ulcers showed only moderate inflammation or hyaline necrosis of the tips of some villi. The submucosa was greatly thickened by edema fluid and masses of fibrin, and contained relatively few leukocytes and histiocytes. The walls of several arteries just inside the muscularis had partly necrotic walls. Brunner's glands were present as small lobuli; because of the swelling of the submucosa they occupied only its superficial part. Sometimes they occupied the base of the ulcers. Here as well as in other places, where the overlying mucosa was intact, mitotic divisions were found in the glandular epithelium, though not in great numbers. Here and there leukocytes and desquamated epithelial cells were found in the lumina of the glands.

Case 2

The body was that of a colored male, 37 years old. Autopsy was performed 60 minutes after death. Anatomical diagnoses: cancer of the prostate with metastases in the lungs; bilateral hydronephrosis. The duodenum was without pathological changes.

Microscopical Examination of the Duodenum. The mucosa was without pathological changes. Brunner's glands were unevenly distributed, forming sometimes small lobuli, in other places large accumulations of glandular tissue.

The number of enterochromaffin cells varied from place to place but was not great. The protoplasm of the glandular epithelium was often divided into basal and more superficial parts by a darker staining band of protoplasm.[4] Many cells contained very minute, pale-staining

granules. Also a narrow band of protoplasm at the base of most cells stained more deeply. An occasional mitosis was found in the glandular epithelium.

Case 3

The patient was a colored female, 68 years old, upon whom autopsy was performed 60 minutes after death. Anatomical diagnoses: contracted kidneys; hypertrophy of the heart; pulmonary infarct; gastric ulcer. The duodenum was without pathological changes.

Microscopical Examination of the Duodenum. The mucosa was normal. In the proximal part of the duodenum there was a large complex of Brunner's glands; in the rest, only small lobuli. The glandular epithelium showed sometimes the same division of its protoplasm as described for case 2. Other cells were filled with minute, pale-staining granules and still others were large and swollen and contained flattened pyknotic nuclei, which were lying at the bases of the cells. There were very few enterochromaffin cells. An occasional mitosis was found in the glandular epithelium.

Case 4

The body was that of a colored male, 60 years old. Autopsy was performed 40 minutes after death. Anatomical diagnoses: coronary sclerosis; extensive fibrosis of the myocardium; ascites; bilateral hydrothorax. The mucous membranes of the stomach and the duodenum appeared to be somewhat swollen.

Microscopical Examination of the Duodenum. The tips of a few villi showed hyaline necrosis; also the scar of a healed, superficial erosion was seen. Brunner's glands were present as large accumulations of glandular tissue. There were very few enterochromaffin cells in the glands. The structure of the glandular epithelium varied widely. Whereas some cells showed the typical aspect of those of mucous glands—large, pale-staining cells with nuclei flattened at the bases of the cells—others were smaller with an oval to rounded nucleus and horizontal strips of dark-staining protoplasm at the base and more or less halfway between the base and the apex of the cell. In the glandular lumina small droplets, possibly of precipitated secretion, were found.

In the glandular epithelium mitotic divisions were very numerous; in some areas 4 to 7 mitoses could be found in one high-power field. They occurred in both types of cells.

Case 5

The patient was a colored male, 55 years old, upon whom autopsy was performed 45 minutes after death. Post-mortem diagnoses: granulating ulcerative colitis; small erosion at the pyloric ring. The duodenum did not show pathological changes.

Microscopical Examination of the Duodenum. The mucosa showed no pathological changes. Brunner's glands were well developed. Be-

tween the tubuli small groups of plasma cells were found. In some places there were many enterochromaffin cells in the glands. The aspect of the glandular epithelium varied as in case 4. An occasional mitosis was found.

Case 6

The body was that of a colored male, 60 years old. Autopsy was performed 60 minutes after death. Anatomical diagnoses: gangrene (diabetic) of both feet; bleeding duodenal ulcer 3 cm. from the pylorus; atrophy of the pancreas; esophagitis.

Microscopical Examination of the Duodenum. Sections from the region between the pylorus and the ulcer showed a few minute spots of hyaline necrosis in the propria mucosae, sometimes still covered with epithelium. Everywhere else the mucosa and the surface epithelium were intact. Brunner's glands were well developed and occupied the whole breadth of the submucosa. The nuclei of the epithelial cells of the glands were oval or rounded and the deep-staining zone of protoplasm at the bases of the cells was well visible. In several places mitotic divisions were found. The ulcer had penetrated into the pancreas. Its edges were steep so that Brunner's glands bordered directly on the necrotic and infiltration zone. Here also mitotic divisions were easily found, sometimes 2 or 3 in one high-power field. The glands retained their typical aspect; atypical formations as in Robertson's [1] Figure 6b were not seen. There was a small acute ulcer in the neighborhood of the larger one; it had penetrated into the submucosa. Brunner's glands bordering on this ulcer also showed a few mitoses.

Case 7

The patient was a colored female, 33 years old, upon whom autopsy was performed 10 minutes after death. Anatomical diagnoses: diffuse serofibrinous peritonitis; postpartum endometritis; necrotic submucous leiomyoma of the uterus. The duodenum was removed immediately after the opening of the abdomen. In the proximal part was a small shallow ulcer.

Microscopical Examination of the Duodenum. The lesions found in the mucosa differed only in extent and degree. In some places only stumps of villi remained. They were covered by epithelium which was lower and stained lighter than usual and did not show a striated border. The normal stroma of the villi was replaced by a hyaline, pink-staining mass, which did not contain nuclei, except those of polymorphonuclear leukocytes. In other places the epithelium had disappeared and hyaline necrosis extended to the muscularis mucosae. The small ulcer had penetrated into the submucosa; it was surrounded by more superficial lesions as described before. The submucosa in the region of the ulcer

was swollen and contained large masses of fibrin and many leukocytes. The normal structures—glands, vessels and nervous tissue—were widely separated; there were but few fibrocytes. Brunner's glands were well developed. In the areas where the erosions and ulcers were found, the glandular epithelium stained more deeply than normal and the cells were smaller than usual. There were few enterochromaffin cells. At some distance from the erosions and from the ulcer, mitoses could be found easily, but in their immediate neighborhood they were present everywhere in great numbers so that sometimes 10 or more mitotic division figures could be counted in one high-power field. Even in glands lying in the base of the ulcer and bordering directly on necrotic tissue, mitoses were found.

Case 8

The body was that of a colored female, 16 years old. Autopsy was performed 40 minutes after death. Anatomical diagnoses: multiple congenital malformations and defects; open ductus Botalli; duodenal ulcer.

Microscopical Examination of the Duodenum. Except for the ulcer, the mucosa was intact. There was diffuse hyperplasia of Brunner's glands. The glandular epithelium had pale-staining protoplasm; the nuclei were flattened at the bases of the cells. The ulcer had penetrated into the pancreas; the edges showed partial epithelialization; there was extensive fibrous reaction. Brunner's glands at the edge of the ulcer showed a few mitotic divisions.

Discussion

As appears from the case reports, we found signs of proliferation in Brunner's glands in 8 of 24, or in one-third of all cases. In cases 1, 6, 7 and 8 there was ulceration of the duodenum and in case 4 a few minute and superficial erosions. Whereas in cases 2, 3, 5 and 8 only a few mitoses were found, they were present in greater numbers in cases 1 and 6, and in cases 4 and 7 they abounded. The few mitoses found in cases 2, 3 and 5 probably represent regeneration after physiological loss of cells, though it is of course also possible that a very slowly developing hyperplasia, caused by some unknown factor, may have been under way. In case 8 the chronicity of the ulcer was perhaps the reason why only a few mitoses were found. Brunner's glands were separated from the areas of necrosis and infiltration by a layer of dense connective tissue and probably an equilibrium between destruction and regeneration of glandular epithelium had been reached. It is difficult to explain the presence of so many mitoses in case 4. It is hard to believe that the few, very small, superficial necroses were the cause. In cases

1, 6 and 7 it was the presence of acute ulceration with the destruction of many cells which caused regenerative proliferation. It should be emphasized that even glands partially surrounded by necrotic tissue showed many mitoses, a phenomenon often observed in the epithelium of the crypts of Lieberkühn in bacillary dysentery. It is interesting to note that in cases 6 and 8, in which the ulcers had penetrated into the pancreas, mitoses were found in the smaller intralobular ducts and the so-called centro-acinar cells.

Though from the statistical point of view our series of cases is very short as compared with Robertson's [1] 1000 cases, our percentage of cases in which mitoses were found is so high (33 1/3 per cent as compared with 0.2 per cent), and especially the number of mitoses in individual cases is so great, that in our opinion the factor of chance can be excluded. That the difference between our results and those of Robertson is attributable to racial differences, our material being derived exclusively from colored people, is not probable. Nowhere have we found indications in the literature that such differences do exist, though in other respects there are many.[5] Another possible explanation is that the material used by Robertson was not suitable for these investigations. That this explanation is probably the right one is proved by his photomicrographs. Most of them show well advanced post-mortem changes. We have several times pointed out that such material is not suitable for the finding of mitotic divisions or for delicate histological work.[6, 7] In morphological pathology, as in every other branch of scientific investigation, only appropriate methods will give good results.

Summary

In 8 of 24 duodena examined microscopically, signs of proliferative activity, *i.e.*, mitotic divisions, were found in Brunner's glands. In 4 cases an occasional mitosis was found, in 2 cases they were in sufficient numbers to be found easily, and in 2 other cases mitotic divisions literally abounded. In the presence of acute inflammatory conditions the epithelium of Brunner's glands reacts in the same way as glandular epithelium in other parts of the body.

REFERENCES

1. Robertson, H. E. The pathology of Brunner's glands. *Arch. Path.*, 1941, 31, 112–130.
2. Maximow, A. A., and Bloom, W. A Textbook of Histology. W. B. Saunders Co., Philadelphia & London, 1942, ed. 4, p. 37.
3. Mallory, F. B. Pathological Technique. W. B. Saunders Co., Philadelphia & London, 1938, p. 54.
4. Macklin, C. C., and Macklin, M. T. The Intestinal Epithelium. In: Cowdry, E. V. Special Cytology. Paul B. Hoeber, Inc., New York, 1928, 1, 188.

5. Lewis, J. H. The Biology of the Negro. University of Chicago Press, Chicago, 1942.
6. Hartz, P. H., van der Sar, A., and van Meeteren, A. The occurrence of mitotic divisions in glomeruli in glomerulonephritis and malignant sclerosis. *Am. J. Path.*, 1941, **17**, 563–570.
7. Hartz, P. H., and Hugenholtz, M. J. Mitotic activity in uterine leiomyomas. *Am. J. Clin. Path.*, 1942, **12**, 523–524.

[*Illustrations follow*]

DESCRIPTION OF PLATES

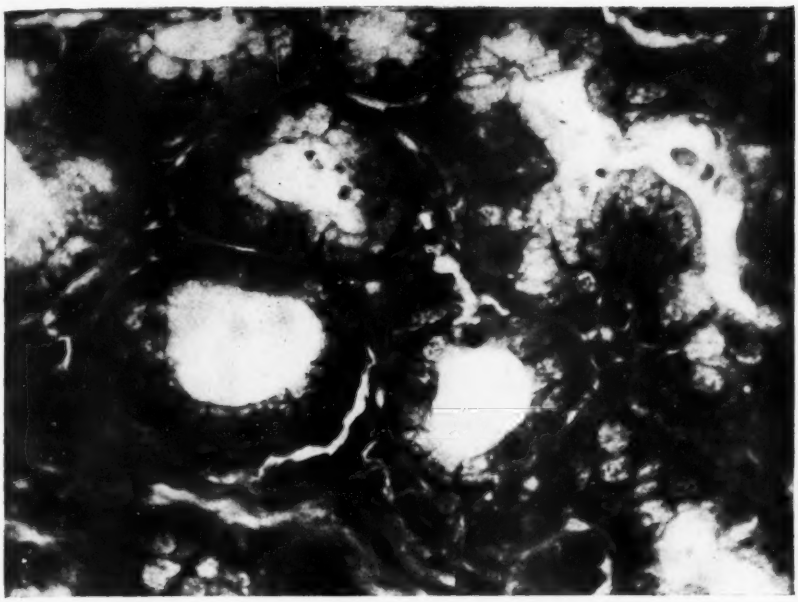
PLATE 166

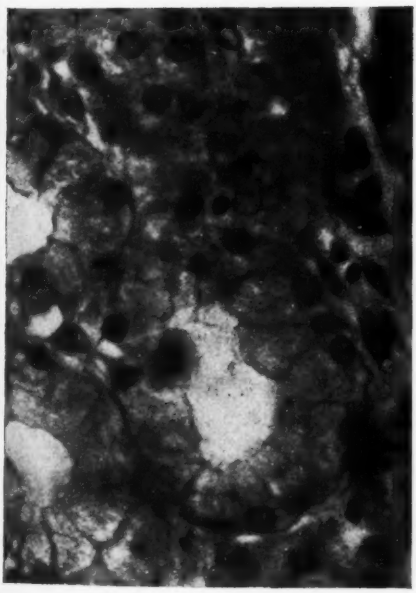
FIG. 1. Case 4. Four mitotic figures are present in a relatively small field. Fixation in Orth's fluid. × 621.

FIG. 2. Case 6. Two mitotic figures are found in the field photographed. Fixation in Bouin-sublimate fluid. × 621.

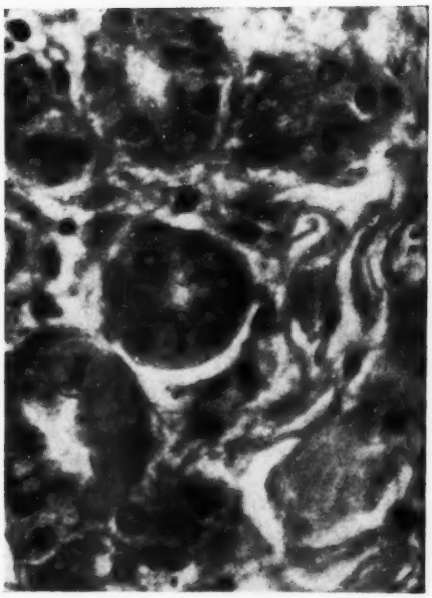
FIG. 3. Case 7. Five mitoses are present in the area illustrated. Fixation in Orth's fluid. × 621.

1





2



3

Hartz and van der Sar

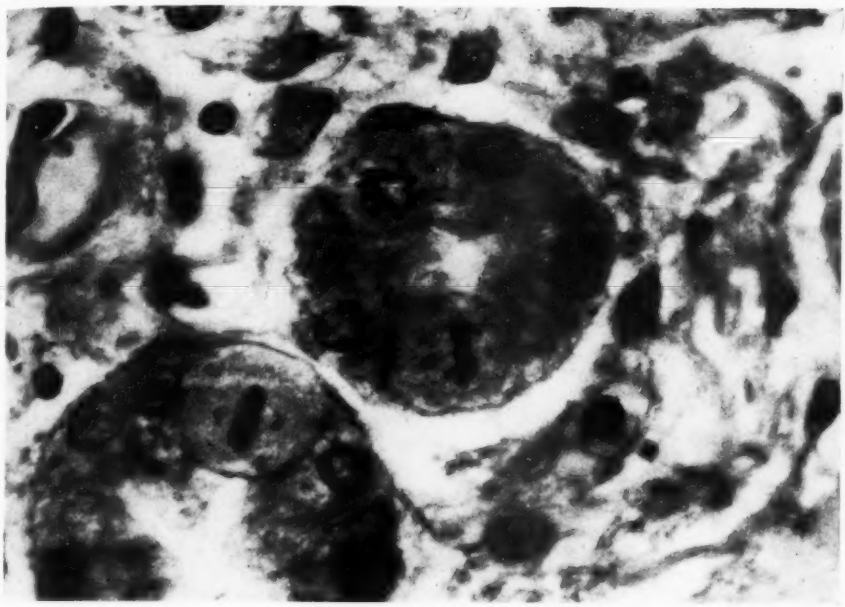
Proliferative Activity in Brunner's Glands

PLATE 167

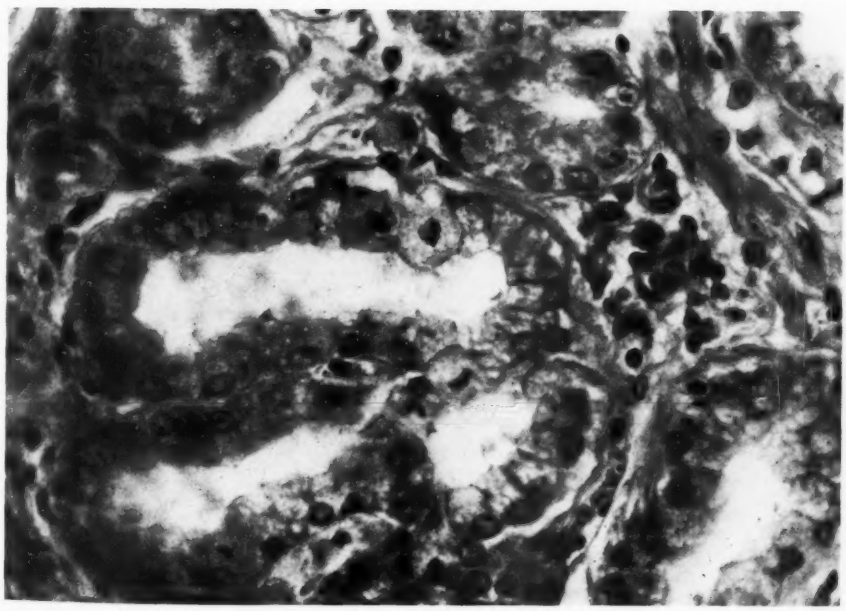
FIG. 4. Case 7. A portion of the same field as in Figure 3 is shown at a higher magnification. × 1300.

FIG. 5. Case 7. Four mitoses are shown. Fixation in Orth's fluid. × 621.

4



5



Hartz and van der Sar Proliferative Activity in Brunner's Glands

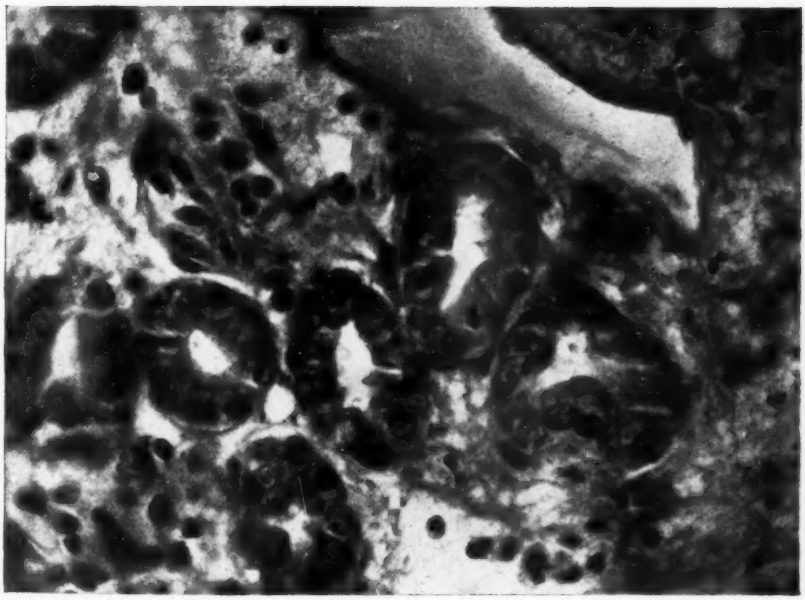
PLATE 168

FIG. 6. Case 7. Brunner's glands in the base of an ulcer. Two mitoses are shown in one tubule. Fixation in Orth's fluid. × 303.

FIG. 7. Case 7. A portion of the field of Figure 6 is shown at a higher magnification. The dark-staining zones of the protoplasm of the epithelial cells are shown. × 621.

6

7



Hartz and van der Sar Proliferative Activity in Brunner's Glands

STUDIES ON THE EARLY CHANGES IN THE LIVERS OF RATS TREATED WITH VARIOUS TOXIC AGENTS, WITH ESPECIAL REFERENCE TO THE VASCULAR LESIONS

I. The Histology of the Rat's Liver in Urethane Poisoning *

L. Doljanski, M.D., and A. Rosin, M.D.†
(From the Department of Experimental Pathology, The Hebrew University, Jerusalem, Palestine)

It is generally accepted that retrogressive changes in the hepatic parenchyma may be caused either by direct action of exogenous or endogenous noxious agents on liver cells, or by insufficient blood supply following the total or partial occlusion of the afferent blood vessels. It has been repeatedly suggested also (Rössle,[1] Schürmann and MacMahon,[2] Eppinger, Kaunitz and Popper [3]) that alterations in the structure and function of the lining of the sinusoidal capillaries may lead to pathological changes in the liver parenchyma. Nevertheless, the rôle played by capillary damage in the genesis of lesions of the liver cells is not yet established.

It is certain that the obstruction of an afferent blood vessel can produce necrosis in the area supplied by it. It is conceivable, but it has not been finally demonstrated, that certain endogenous or exogenous toxic substances may interfere with the very specialized metabolic processes taking place in the liver cells and may thus selectively damage them. But it is also highly suggestive that for the life of the liver cell the functional integrity of the lining of the sinusoidal capillaries is essential and that its alterations can provoke damage to the parenchymatous cells.

The following questions present themselves on approaching the problem of the pathogenesis of the retrogressive changes in liver parenchyma induced by various toxic agents:

1. Are there specific liver cell poisons, *i.e.*, substances capable of bringing about damage to the liver cells directly and selectively?

2. Can substances known to be capillary poisons, in addition to the capillary injury, provoke degenerative changes in the liver cells, and, if so, what is the relation between the capillary damage and the degenerative processes in the parenchyma?

3. What is the relative rôle of capillary injury and of primary damage to liver cells in the development of necrobiotic changes in the liver parenchyma resulting from the action of various external and internal noxious agents?

* Received for publication, December 9, 1943.
† Working under The Cancer-Laboratories Fellowship.

The course of the investigations to be described is indicated by the questions outlined above. They represent a comparative study of the action on the liver of poisons of two different types: capillary poisons and so-called liver cell poisons. Final conclusions can be drawn only after comparing and contrasting the mode of action of the various toxic agents to be used. Since histopathogenetic analysis of the action of the diverse toxic substances on liver can effectively be undertaken only before intense damage has developed, our investigations deal mainly with the early stages of the lesions. This, the first paper of a series, is concerned with the changes induced in the liver by urethane.

Material and Methods

Urethane (ethyl carbamate) is a drug with definite vascular action. It produces dilatation of the capillaries, causing an increase in the permeability of the capillary lining (Krogh and Harrop [4]). According to Landis [5] the increased permeability of the capillary wall is not due to the dilatation of the vessel, but in the first place to the toxic effect of urethane on endothelial cells. The toxic effect of urethane has also been demonstrated on *Paramecium caudatum* and on developing eggs of marine animals by Landis, and on fibroblasts in tissue cultures by Geiersbach.[6] Little is known of the action of urethane on the liver. Sollmann [7] mentioned vacuolization of liver cells after administration of urethane. Whipple and Speed [8] found that the administration of large doses of urethane decreased the excretion of phenolphthalein through the bile.

Our observations were made on 25 young albino rats weighing between 70 and 120 gm. The urethane was injected by the intraperitoneal route in a 10 per cent aqueous solution, in single or repeated doses. Animals which received high doses succumbed at varying intervals after injection. The others were sacrificed at specified times.

Pieces of liver were fixed in Carnoy's fluid, Zenker's solution and in a 4 per cent solution of formaldehyde, and blocked in celloidin-paraffin. Sections, 6 μ in thickness, were stained in a variety of ways: hematoxylin and eosin, Giemsa's method, iron-hematoxylin, Mallory's aniline blue collagen stain and Feulgen's method. For identification of fat, frozen sections were stained with Sudan III.

Results

A. Effect of a single dose of urethane

The rats of this series were injected intraperitoneally with doses of 1 to 5 cc. of a 10 per cent solution of urethane per 100 gm. rat. Doses of 3 cc. and more of the 10 per cent solution of urethane will generally

kill the animals in a few minutes. Death may also occasionally occur after doses of 1.5 to 2 cc. In that case the animals die some hours after injection. Animals which did not die were sacrificed 5 to 24 hours after injection.

1. *Rats Which Died 5 Minutes after Administration of 3 to 5 cc. of a 10 Per Cent Urethane Solution.* Macroscopically, the liver appeared enlarged and dark red; its lobular pattern was slightly exaggerated. The outstanding change in the liver revealed by microscopical examination was edema around the portal veins (Fig. 1). The portal veins were surrounded by ring-shaped homogeneous areas which stained uniformly pink with eosin. The accompanying lymph vessels were greatly dilated. The liver cells were pressed back from the vessels by the extravasated fluid. The blood sinusoids in the peripheral parts of the lobules were engorged with red cells and showed no signs of alteration. In the central zone of the lobules the walls of the blood sinusoids were often detached from the liver cell cords. The capillaries lay free in the sinusoidal clefts. The distended pericapillary spaces contained red cells, and sometimes pink granular material. The liver cells were well preserved, but in the central parts of the lobules they showed here and there slight vacuolization.

2. *Rats Which Died 3 Hours after Administration of 1.5 cc. of a 10 Per Cent Urethane Solution.* The damage to the portal veins was more accentuated. There were not only extravasations of plasma, but also of formed elements of the blood (Fig. 2). Large areas of hemorrhage were seen surrounding many of the branches of the portal veins. In the walls of the portal veins numerous red cells were found between the collagen fibers. The changes in the blood sinusoids were similar to those described above and took place always in the central parts of the lobules. The liver cells were definitely vacuolated, especially in the central parts of the lobules, the vacuoles containing only occasional fat droplets; in the periphery the liver cells showed no, or very slight, vacuolization. The nuclei of the liver cells presented no changes.

3. *Rats Which Died 7 Hours after Administration of 1.5 cc. of a 10 Per Cent Urethane Solution.* The portal vessels and larger collecting veins were often surrounded by areas containing edema fluid, granular material and red blood corpuscles (Fig. 3). In addition, there was most intensive damage to the walls of the blood sinusoids, especially in the central parts of the lobules. They were to a great extent separated from the liver cell cords, and the pericapillary spaces contained large quantities of eosinophilic granular material and red blood cells (Fig. 4). The continuity of the capillary wall was frequently interrupted, and its fragments could be seen floating in the sinusoidal

clefts, mixed with the eosinophilic granular masses and red blood cells. The Kupffer cells were frequently detached and pyknotic. In the zones of the intensive capillary damage, namely, in the central parts of the lobules, the liver cells were finely vacuolated and often dissociated (Fig. 5); but in the periphery they showed no abnormalities.

4. *Rats Sacrificed 5 and 24 Hours after Administration of 1 to 2 cc. of a 10 Per Cent Urethane Solution.* Macroscopically, the liver presented a normal aspect or was slightly congested. Microscopically, the cytoplasm of the liver cells was often vacuolated, the vacuolization being more evident in the central than in the peripheral parts of the lobules. In the livers of animals sacrificed after 24 hours some of the vacuoles contained fat. The blood sinusoids appeared normal; their walls adhered to the liver cell cords. Kupffer cells were sometimes prominent. The walls of the portal veins were often edematous and their lining endothelium was missing in various parts. Only occasionally branches of the portal vein showed ring-shaped hemorrhages.

Summary

Animals dying a few minutes after injection of a single dose of urethane showed extravasation of blood plasma around the branches of the portal veins and marked pericapillary edema. In rats surviving for several hours, damage to the portal veins and blood sinusoids was more marked. The walls of the portal veins were infiltrated with erythrocytes, and extensive extravasation of formed elements of the blood through the vascular walls sometimes occurred. The walls of the blood sinusoids, especially in the central parts of the lobules, were often separated from the liver cell cords and sometimes broken. The pericapillary spaces often contained granular material and red blood cells. The liver cells, especially in the central parts of the lobules, showed vacuolization and were sometimes dissociated; in the periphery they showed very slight changes or none. Rats sacrificed after a single nonlethal dose of urethane showed changes of the same type, but much less pronounced.

B. Effect of Repeated Administration of Urethane in Increasing Doses

A 10 per cent urethane solution was used; the beginning dose was 0.5 cc.; the dose was increased daily by 0.1 cc. until death occurred. The animals died after 4 to 14 injections.

Macroscopically, the livers of animals in this series were slightly enlarged and showed congestion. Microscopically, the changes of the portal veins in the livers of animals dying after 4 to 5 days were, in

comparison to the changes described above, not very marked and consisted of mild edema and slight thickening and homogenization of the vessel wall. Occasionally the intimal endothelium was absent, and small denuded areas were found. Extravasation of formed elements of the blood occurred only seldom. There were but very slight changes in the blood sinusoids. They were generally engorged with red blood cells; their walls almost everywhere were well preserved and closely applied to the liver cell cords. The Kupffer cells were prominent; their protoplasm was often markedly eosinophilic and contained numerous chromatin clumps. The liver cells were often vacuolated, particularly in the central parts of the lobules. The presence of fat in the vacuoles generally could not be demonstrated. The nuclei of liver cells were very variable in size; binuclear cells were numerous. Mitoses were frequent.

In experiments of longer duration (11 to 14 days) the changes became more accentuated. The outstanding feature of the damage in this stage of urethane poisoning was the pronounced change in the central parts of the lobules (Fig. 6). Here the walls of the blood sinusoids were often detached from the liver cell cords, and their continuity frequently interrupted. The capillary lumina contained either red blood cells or eosinophilic granular material, sometimes also found in the pericapillary spaces. The extravasation of plasma and formed elements of the blood from the capillary spaces led to the formation of blood "lagoons." The Kupffer cells were prominent and frequently detached and laden with yellow pigment, fragments of chromatin and a few red cells. Their protoplasm was often markedly eosinophilic. The liver cells in the central parts of the lobules, in the zones of the most accentuated capillary damage, were markedly dissociated, atrophic, and in some places there was a disappearance of liver cells. They were generally highly vacuolated. The presence of fat could, however, seldom be demonstrated. In the cytoplasm of the liver cells, red blood corpuscles, singly or in groups, could be found, generally enclosed in a vacuole. The conglomerates of blood corpuscles often reached considerable size and then pushed the nuclei of the liver cells aside. In the course of time the red corpuscles became fused, at first into masses of reticular structure in which individual blood corpuscles were still occasionally recognized, and finally into massive hyaline balls (Fig. 7). The liver cells containing erythrocytes were enlarged. The nuclei of the liver cells were generally well preserved; their size was variable; sometimes they were of dense structure and sometimes pale. Mitotic figures were frequent.

In some rats (experiments of 5 days' duration) many liver cells

contained a rounded body lying near the nucleus and staining deep blue with hematoxylin (Fig. 8). The size of these bodies was generally one-fourth to one-half of that of the nucleus. They were sometimes homogeneous; sometimes they showed a dark blue periphery, pale center and a network of granular or threadlike structures. They gave a positive Feulgen reaction. These structures indubitably were remnants of second nuclei—a product of their disintegration. Clara[9] noted similar findings in the livers of rabbits poisoned with phosphorus. According to Clara the disintegration of one nucleus in binucleated liver cells mediates the re-establishment of the normal nucleus/cytoplasm ratio which had been altered during the process of regeneration.

Summary

Animals receiving repeated and increasing doses of urethane show relatively slight damage to the portal vessels, manifested by mild edema and homogenization of the vessel wall and occasional endothelial lesions. Changes of the blood sinusoids appear only after prolonged administration of the drug and are limited almost entirely to the central parts of the lobules, where the lining of the sinusoidal capillaries is often detached from the liver cells and sometimes broken. The damage of the capillaries leads to more or less extended extravasation of blood in the center of the lobule with formation of blood "lagoons." The liver cells in the zones of the strongest capillary damage are markedly vacuolated, often dissociated, and sometimes there is disappearance of the liver cells. The liver cells may contain red blood cells, generally fused to spherical masses.

Discussion

The outstanding findings in the livers of animals receiving single or repeated doses of urethane are the damage to portal veins and sinusoidal capillaries, manifested by lesions of the vessel walls which become permeable to blood plasma or to the formed elements of the blood according to the degree of damage.

There are, however, not only vascular and capillary lesions, but also alterations of the parenchymal cells. Compared with that of the blood vessels, the damage of the hepatic cells is only of slight degree and is in the first place manifested by intracellular edema (hydropic degeneration); prolonged administration of urethane may lead to atrophy and disappearance of the liver cells. All liver cell changes take place in the central parts of the lobules, *i.e.*, in zones of the most evident capillary damage. In the peripheral region the liver cells are normal. True necrosis of liver cells was in no case observed.

The characteristic feature of the changes in the liver cells in ure-

thane poisoning is the hydropic degeneration. Hydrops of liver cells has been observed in several pathological conditions, particularly in various kinds of poisoning. The opinion has been expressed that this is caused by the intake of fluid by the liver cells, and that this increased intake occurs when the fluid of the blood in unusual quantities and probably of unusual composition passes from blood vessels into the intracellular spaces.

Investigations on perfused liver provide an experimental basis for this view. The endothelial damage taking place during the perfusion inevitably leads to capillary failure followed by extravasation of fluid. The vacuolization of liver cells is the immediate response to this event. Raum[10] has perfused entire animals with 0.6 per cent NaCl solution. After 2½ to 3 hours intense vacuolization of liver cells could be observed. He considered that the vacuolization of liver cells is a reversible phenomenon due to the surplus of fluid offered. Von Skramlik and Hünermann[11] perfused isolated livers of dogs with Ringer's solution and observed that 2 hours after the beginning of the experiments the vacuolization of liver cells appears. Detachment of the capillary membrane from the liver cell cords—pericapillary edema—could also be observed. Milletti,[12] in similar experiments, saw the vacuolization of the liver cells as early as 10 minutes after the perfusion had started.

The fact that in urethane poisoning altered liver cells are frequently encountered, which contain erythrocytes or their disintegration products, is further evidence that in the genesis of the parenchymal changes in urethane poisoning the capillary failure plays a rôle. The ingestion of erythrocytes by liver cells is obviously possible only where the elements of the extravasated blood come in direct contact with the parenchymal cells.

Ingestion of the red blood corpuscles by the liver cells was first described in dogs after injection of hemoglobin by Browicz.[13] Some time later the same phenomenon was reported by Vereecke,[14] whose observations were made on dogs which received injections of curare, distilled water, or peptone. Rössle[15] saw erythrocytes within liver cells in a case of human hemochromatosis. He expressed the belief that there exists an infectious-toxic disturbance of the capillaries leading to changes in the permeability of the capillary wall. This is followed by extravasation of blood corpuscles which then come in direct contact with the parenchymal cells, and become phagocytized by the latter. Heinrichsdorff[16] reported ingestion of red blood corpuscles by liver cells in cases of jaundice. He assumed that in this condition the capillary damage occurs simultaneously with the damage of the liver cells. Gräff[17] described infiltration of liver cells by red blood corpuscles in guinea-pigs poisoned with Amanita toxin. Weatherford[18] observed a

similar phenomenon in dogs in anaphylactic shock. He stated that: "The evidences of increase of capillary permeability, disorganization of the sinusoidal endothelium and parenchymatous necroses detailed in severe anaphylactic shock in the dog make it quite possible that erythrocytes may pass either by diapedesis through the endothelium or directly into the injured hepatic cells."

In analyzing the preceding experimental findings, the following statements concerning the correlation of the liver cells and capillary injury in urethane poisoning can be made:

1. The vascular changes in urethane poisoning predominate to a high degree over the parenchymal injuries.

2. Vascular and liver cell changes are correlated temporally and locally. The changes in the parenchymal cells and the vascular injury appear practically at the same time and unmistakable changes in the liver cells occur only in places where the vascular changes are evident.

3. The character of the parenchymal injuries in urethane poisoning definitely indicates that they take place in an internal environment altered by the breakdown of the normal blood-tissue barrier.

The evidences cited, although highly suggestive that parenchymal changes are causally related to vascular damage, are not sufficient to exclude a direct action of urethane on the liver cells. Pending further experiments, any speculation concerning the causal genesis of liver cell damage in urethane poisoning will remain inconclusive.

Summary

The effect of single and multiple injections of urethane on the liver of the white rat has been studied in respect to alterations of parenchymal cells and vascular lesions. Urethane in large amounts produces very pronounced damage of portal veins and of sinusoidal capillaries, which leads to extravasation of plasma and formed elements of the blood through the vessel wall. The changes in the parenchyma are comparatively slight and consist of vacuolization of the liver cells (hydropic degeneration) combined with occasional intake of red cells. After prolonged action of urethane there is atrophy and disappearance of the liver cells in the central parts of the lobule. Although strongly suggesting that the parenchymal lesions are causally dependent upon vascular injury, the evidence obtained was insufficient to exclude a direct action of urethane on liver cells.

REFERENCES

1. Rössle, R. Entzündungen der Leber. In: Henke, F., and Lubarsch, O. Handbuch der speziellen pathologischen Anatomie und Histologie. J. Springer, Berlin, 1930, 5, pt. 1, 243–505.

2. Schürmann, P., and MacMahon, H. E. Die maligne Nephrosklerose, zugleich ein Beitrag zur Frage der Bedeutung der Blutgewebsschranke. *Virchows Arch. f. path. Anat.*, 1933, **291**, 47–218.
3. Eppinger, H., Kaunitz, H., and Popper, H. Die seröse Entzündung. J. Springer, Wien, 1935.
4. Krogh, A., and Harrop, G. A. Some observations on stasis and edema. *J. Physiol.*, 1920–21, **54**, CXXV–CXXVI.
5. Landis, E. M. Micro-injection studies of capillary permeability. I. Factors in the production of capillary stasis. *Am. J. Physiol.*, 1927, **81**, 124–142.
6. Geiersbach, U. Über den Einfluss der Narkose (Urethan) auf Gewebekulturen. *Arch. f. exper. Zellforsch.*, 1939, **23**, 210–219.
7. Sollmann, T. H. A Manual of Pharmacology and Its Applications to Therapeutics and Toxicology. W. B. Saunders Co., Philadelphia, 1934, ed. 5.
8. Whipple, G. H., and Speed, J. S. Liver function as influenced by anesthetics and narcotics. *J. Exper. Med.*, 1915, **21**, 203–212.
9. Clara, M. Über den Bau der Leber beim Kaninchen und die Regenerationserscheinungen an diesem Gewebe bei experimenteller Phosphorvergiftung. *Ztschr. f. mikr.-anat. Forsch.*, 1931, **26**, 45–172.
10. Raum, J. Künstliche Vacuolisirung der Leberzellen beim Hunde. *Arch. f. exper. Path. u. Pharmakol.*, 1892, **29**, 353–363.
11. von Skramlik, E., and Hünermann, T. Die überlebende, künstlich, durchströmte Leber im histologischen Bild. *Ztschr. f. d. ges. exper. Med.*, 1920, **11**, 349–366.
12. Milletti, M. Modificazioni citologiche della cellula epatica in seguito a perfusione del fegato con soluzione di Ringer-Locke normale e addizionata di gelatina. *Boll. Soc. ital. biol. sper.*, 1936, **11**, 404–405.
13. Browicz. Meine Ansichten über den Bau der Leberzelle. *Virchows Arch. f. path. Anat.*, 1902, **168**, 1–22.
14. Vereecke, A. Sur une infiltration spéciale des éléments parenchymateux du foie dans diverses conditions expérimentales. *Arch. de Pharmacod.*, 1895, **2**, 47–95.
15. Rössle, R. Über Phagocytose von Blutkörperchen durch Parenchymzellen und ihre Beziehung zum hämorrhagischen Odem und zur Hämochromatose. *Beitr. z. path. Anat. u. z. allg. Path.*, 1907, **41**, 181–222.
16. Heinrichsdorff. Zur Histogenese des Ikterus. *Virchows Arch. f. path. Anat.*, 1924, **248**, 48–90.
17. Gräff, S. Knollenblätterschwamm-(extrakt)-Vergiftung beim Tier. *Verhandl. d. deutsch. path. Gesellsch.*, 1927, **22**, 284–288.
18. Weatherford, H. L. The influence of anaphylactic shock on the finer structure of the liver in the dog. *Am. J. Path.*, 1935, **11**, 611–629.

[*Illustrations follow*]

DESCRIPTION OF PLATES

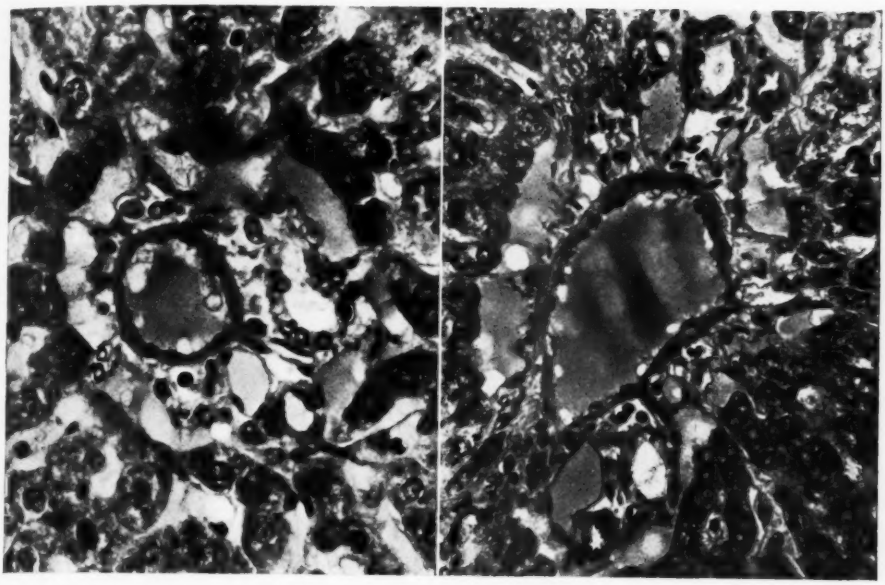
PLATE 169

(All figures taken from hematoxylin and eosin preparations.)

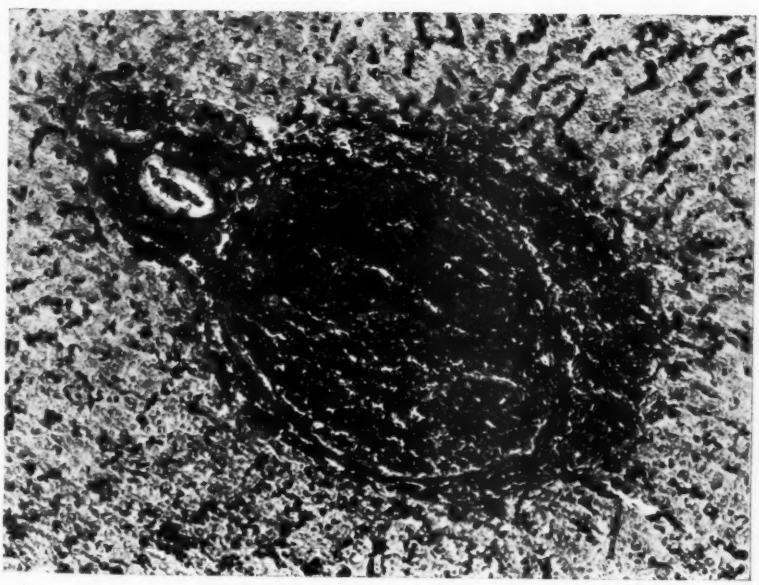
FIG. 1. Two portal veins from rat 60, which died 5 minutes after the administration of 3 cc. of 10 per cent urethane solution, showing the marked edema around the veins. × 580 and × 410.

FIG. 2. From rat 119, which died 3 hours after the administration of 1.5 cc. of 10 per cent urethane solution. Extensive hemorrhage is shown about a portal vein. × 190.

1



2



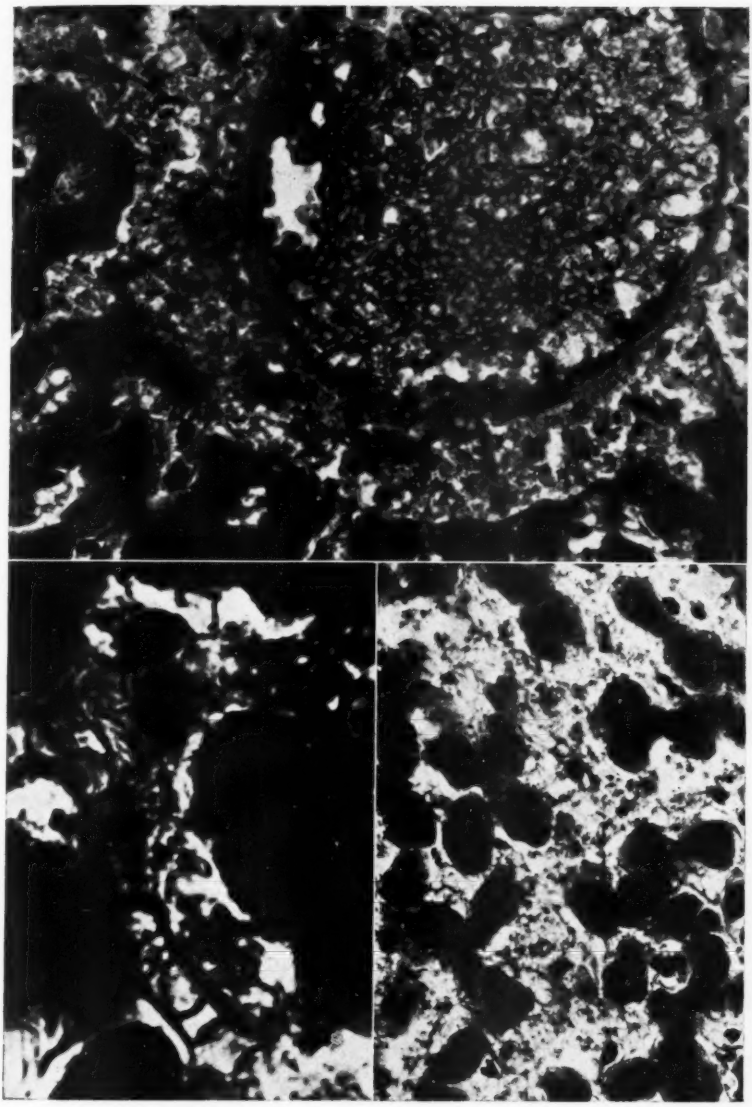
Doljanski and Rosin Liver in Urethane Poisoning

PLATE 170

FIG. 3. From rat 64, which died 7 hours after the administration of 1.5 cc. of 10 per cent urethane solution. There is an extravasation of both plasma and the formed elements of the blood through the wall of the portal vein. × 680.

FIG. 4. From the same animal as Figure 3. Granular material is found in the distended pericapillary spaces. × 1900.

FIG. 5. From the same animal as Figures 3 and 4. Dissociation of liver cells in the central part of the liver lobule. × 540.



3

4

5

Doljanski and Rosin Liver in Urethane Poisoning

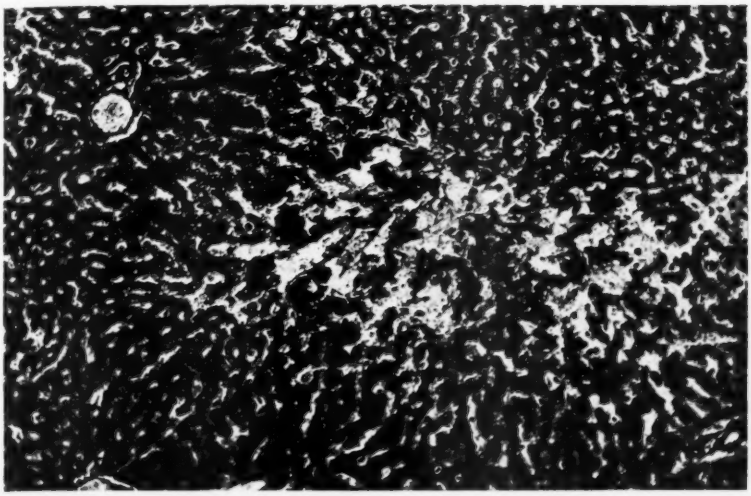
PLATE 171

FIG. 6. From rat 131, which died 13 days after repeated administration of urethane in increasing doses. The changes in the central part of a liver lobule are shown. × 120.

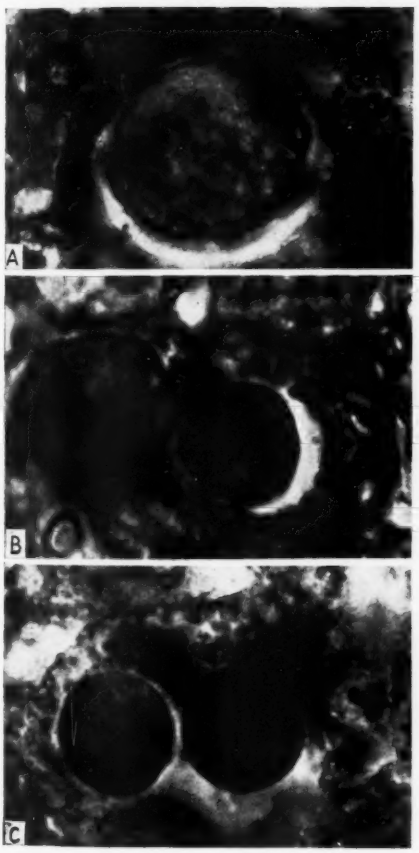
FIG. 7. From rat 130, which died 14 days after the repeated administration of urethane in increasing doses. Conglomerate collections of erythocytes within liver cells are shown in three different stages of disintegration. × 1245, × 1400, and × 1320, respectively.

FIG. 8. From rat 127, which died 5 days after the repeated administration of urethane in increasing doses. Remnants of second nuclei are shown in three binucleated liver cells. × 1320 for all.

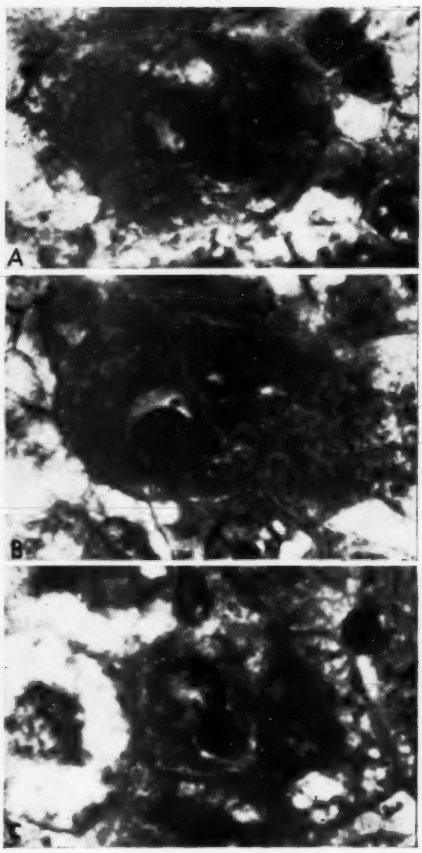
6



7



8



Doljanski and Rosin Liver in Urethane Poisoning

STUDIES ON AMEBOID MOTION AND SECRETION OF MOTOR END-PLATES

IV. Anatomic Effects of Poliomyelitis on the Neuromuscular Mechanism in the Monkey*

Eben J. Carey, M.D.

(From the Department of Anatomy, Marquette University School of Medicine, Milwaukee, Wis.)

A preliminary report has been made[1] of the observations on the early morphologic disappearance of motor end-plates and early appearance of ephemeral projection masses of gold-staining substance in the striped muscle fiber with, or following shortly after, the onset of paralysis in acute poliomyelitis in the monkey. In previous studies by Landsteiner and Levaditi,[2] Flexner and Lewis,[3] Taylor,[4] Penfield,[5] Fairbrother and Hurst,[6] Hurst,[7] Covell,[8] Toomey and Takacs,[9] Sabin and Olitsky,[10] Goodpasture[11] and Howe and Bodian,[12] on the histology of poliomyelitis in the monkey, attention was concentrated on the changes in the central nervous system and on parts of the peripheral nervous system. There have been no studies made of the alterations in the neuromuscular mechanisms. In Hurst's[13] excellent study on the histology of experimental poliomyelitis in the monkey, his observations on muscle are limited to one statement as follows: "Muscles.—Several cases of sarcosporidiosis were found at autopsy." On the other hand, Hassin[14] detected microscopic alterations in epidemic poliomyelitis (Heine-Medin's disease) in human muscle that he considers more important in explaining the symptoms than the alterations in the central nervous system.

The mechanism of action of the virus of poliomyelitis in producing the disappearance of the motor end-plates is unknown. It is assumed that there is an abnormal explosive discharge of the secretion of hypolemmal axonic substance. This gold-staining substance of the axons of the end-plates is assumed to be projected into the myoplasm of the muscle fiber. For some unknown reason this substance projected from the axons does not normally disperse but agglutinates into masses with the accompanying pathologic changes produced by the virus of poliomyelitis. These masses disappear within the first week after their appearance and are not found in all muscles.

The purpose of this paper is to present the complete photomicro-

* These investigations were carried out with the aid of grants for research to the Department of Anatomy of the Marquette University School of Medicine by the Committee on Scientific Research of the American Medical Association, and the National Foundation for Infantile Paralysis, Inc.

Received for publication, December 21, 1943.

graphic evidence, revealed by the gold method, of the alterations in the motor end-plates during the early stages of experimental poliomyelitis in the monkey. Fortunately, the photographs are clear enough without a long, detailed description of the pathologic changes. The exact and clear presentation of these easily verifiable facts necessitates the large number of photographs.

Materials and Methods

The present histopathologic study deals with the results obtained by the intracerebral inoculation of a standard dose of 0.5 cc. of a 5 per cent virulent cord emulsion into 6 monkeys (*Macacus rhesus*). The Armstrong-Lansing strain of poliomyelitis virus was used. Additional studies were made on the changes in the motor end-plates of muscles from 8 monkeys obtained from S. D. Kramer, Michigan Department of Health, Lansing; of muscles from 5 monkeys obtained from J. F. Kessel and F. J. Moore, University of Southern California, Los Angeles; of muscles from 2 monkeys obtained from P. F. Clark and A. F. Rasmussen, University of Wisconsin, Madison, and of muscles from 2 monkeys obtained from H. E. Pearson, School of Public Health, University of Michigan, Ann Arbor. We are deeply indebted to these men for excising the muscles and placing them immediately into bottles surrounded by cracked ice in a thermos jug for shipment by air mail or express to Milwaukee.

It was found by experiment that little or no alteration occurred in the motor end-plates when the muscles were cooled to a temperature of 5 to 8° C. as soon as excised and prior to the onset of rigor mortis. Parts of each muscle were either frozen and sectioned, or fixed and stained by various histologic technics. Changes in fat were studied by osmic acid, Sudan III, and scharlach R, and the neurofibrils by the Bielschowsky method counterstained with iron hematoxylin and van Gieson and with toluidin blue. The best method for the demonstration of the pathologic changes in the anatomic continuity of the epilemmal axon, hypolemmal axons that constitute the motor end-plates, and the organization of a striped muscle fiber was a modified gold method followed by careful teasing of small pieces (2 by 5 mm.) of the impregnated muscle. This report will be limited to the findings obtained by the gold method. This Ranvier gold chloride method was modified by Wilkinson [15] and by me [16] and will not be described in detail in this paper.

The following brief protocols are typical:

Monkey 53 (Marquette Series)

April 19, 1943. Inoculated intracerebrally with 0.5 cc. of virulent cord emulsion of the Armstrong-Lansing strain.

April 23, 1943. Rise of temperature.
April 26, 1943. Complete quadriplegia; etherized; muscles immediately excised and run through gold technic.

Monkey 54 (Marquette Series)

April 19, 1943. Inoculated intracerebrally with the Armstrong-Lansing strain.
April 25, 1943. Rise of temperature.
April 27, 1943. Fine general tremor of weakened muscles of lower extremities and paralysis of those of the upper extremities.
April 28, 1943. Complete paralysis of arms, legs and trunk. Difficulty in respiration. Killed with nembutal. Muscles immediately excised and run through gold technic.

Monkey 490 (Kramer's Series)

Feb. 17, 1943. Inoculated intracerebrally with the Armstrong-Lansing strain.
Feb. 24, 1943. Rise of temperature.
Feb. 27, 1943. Weakness of right forelimb.
Mar. 3, 1943. Complete paralysis of arms and partial paralysis of legs. Exsanguination under chloroform for cardiac puncture. Muscles excised immediately and placed in bottles within iced thermos jug and shipped by air express.
Mar. 4, 1943. Muscles received and run through gold technic.

Monkey C-89 (Clark's Series)

Feb. 6, 1943. Inoculated intracerebrally with the Kessel's McK. strain.
Feb. 18, 1943. Ataxia and tremors.
Feb. 19, 1943. Marked general paresis.
Feb. 20, 1943. Complete flaccid paralysis of arms, legs and trunk. Etherized and exsanguinated. Muscles immediately excised and placed in bottles within iced thermos jug.
Feb. 21, 1943. Muscles received by express and run through gold chloride technic.

Monkey K-334 (Pearson's Series)

Dec. 18, 1942. Inoculated intraperitoneally and intranasally with the Armstrong-Lansing strain.
Dec. 21, 1942. Reinoculated intracerebrally and intranasally with a stool suspension.
Dec. 30, 1942. Complete flaccid paralysis of both legs.
Dec. 31, 1942. Etherized. Muscles of right lower leg excised and immediately put in bottles within iced thermos jug. Shipped by air express.
Jan. 2, 1943. Muscles received and run through gold technic.

Monkey K-338 (Pearson's Series)

April 1, 1943. Inoculated intracerebrally with 2 cc. of a 20 per cent suspension of the Armstrong-Lansing strain.
April 2, 1943. Rise of temperature to 102.2° F.
April 18, 1943. Temperature, 106° F.; tremors, ataxia, ruffled fur, paralysis of the right foreleg, weakness of the back and both hind legs.
April 19, 1943. Temperature, 97.4° F. Complete quadriplegia.
April 20, 1943. Temperature, 90° F.
April 21, 1943. Etherized. Muscles immediately excised and put in bottles within iced thermos jug; shipped to Milwaukee by express.
April 22, 1943. Muscles received and immediately run through the gold chloride technic.

At the autopsies, the usual finding of intense congestion of the meningeal vessels and of the gray matter, especially of the anterior horns of the spinal cord, was noted; on section the nervous tissue was more moist than normal and the cut surface glistening. The affected muscles were intensely hyperemic and more fragile than the normal ones.

Thirty selected muscles from both fore and hind limbs in 2 normal monkeys were prepared by the same gold method applied to the paralyzed muscles and used as controls. More than 3000 teased preparations of the innervation of different muscles were used in this study.

The auriphilous masses of material projected from the degenerating axons were found in certain muscles of 8 of the 23 monkeys studied. These displaced axonic masses were found in greatest abundance in the following muscles: biceps brachii, intercostals, quadriceps extensor femoris, biceps femoris, gastrocnemius, tibialis anterior, sternocleidomastoid, trapezius, pectoralis major and deltoid.

Experimental Results

Histopathology of Motor End-Plates

The motor nerve plates in the relatively normal muscle, quadriceps femoris, in the monkey, varied from the retracted state with wide fronds 15 to 20 μ in diameter (Fig. 1) to the expanded state 30 to 40 μ in diameter. The retracted motor end-plates were usually related to coarse, widely spaced cross striations found in the narrow muscle fibers. There were variations, however, in this relationship. The expanded plates were usually related to fine, closely spaced striations found in the wide muscle fibers. There was a closely graded series of transitional stages in the internal structure of the muscle fibers in relation to motor end-plates of various sizes. The retracted motor end-plate had hypolemmal axon terminals surrounded by an accumulation of Kühne's granules. This mantle of Kühne's granules was either diminished in size or completely depleted in the expanded nerve plates.

With progressive increase of ameboid expansion of the motor nerve plates there was a diminution of Kühne's granules and a corresponding increase in number and fineness of closely spaced cross striations. The strongly contracted muscle fibers had large motor nerve plates with two or more moniliform projections separated by wide spaces. With progressive increase in size of the motor nerve plate and increase of fineness of the dark and light transverse striations, the contracted muscle fiber took a differential stain. This indicated either a physical or chemical change in the contracted muscle fiber. There were many

transitional stages and gradations between the coarsely and finely striated muscle fiber. There was a substantial ameboid protrusion of the processes of the nerve plate in various directions in the muscle substance which increased the surface area of the nerve plate and variability of the muscle striae. This was evidence that during life the muscle striae were not constant and rigid membranes.

In some muscle fibers there was a periodic alternation of fine, closely spaced striations and coarse, widely spaced ones. In other places the muscle substance was coagulated into masses in which no striations were visible with the highest powers of the microscope. This compact muscle substance resembled Zenker's [17] waxy degeneration. From experimental evidence, Wells [18] concluded that these opaque hyaline masses represent a local accumulation of lactic acid. By applying lactic acid to striated muscle *in vitro* and *in vivo* he caused structural changes resembling Zenker's waxy degeneration. The internal structure of the muscle fiber, therefore, appeared to be intimately related to the variable biochemical composition. The variations in the normal structure of the living muscle fiber appeared to be an expression of the changing chemical reactions. There may be a closer association between histologic structure and chemical composition than is now realized.

The differential fiber types were present in the normal muscle (Fig. 1) but they were absent in the paralyzed muscle (Fig. 2). Many of the end-plates on the first day of paralysis were retracted into small ball-like masses (Fig. 2) with shortened axis cylinders and intense affinity for gold. Likewise, on the first day of paralysis, there were a few enlarged granular end-plates that had a weak affinity for gold. Between 20 and 25 per cent of the motor end-plates were absent in the paralyzed muscle on the first day (Fig. 11). Within 2 to 4 days after the onset of paralysis about 50 per cent of the end-plates had disappeared (Figs. 6, 12 to 16, and 27 to 33). During the first 24 hours the paralyzed muscle had definite passive hyperemia of the muscular capillaries and veins. The muscular fibers were prominently identified by the presence of congested intramuscular vessels (Figs. 3 and 4, 7 to 10, and 17). During the first 48 hours the paralyzed muscle had trees of end-plates with variable degrees of retraction and granular fragmentation (Figs. 3 to 5).

Many retracted trees of axons were completely depleted of end-plates by the third or fourth day of paralysis (Fig. 6) and had sharp or bud-like epilemmal terminals in the triceps muscle. The rates of nerve degeneration were unequal in different muscles of the same animal and in different fibers of the same muscle. The weakened biceps

brachii muscle had an innervating tree of retracted ball-like motor end-plates (Fig. 10) whereas the paralyzed triceps muscle had, on the first day of paralysis, an innervating tree with practically complete absence of all of the motor end-plates (Fig. 11).

Observations were made of an unusual histologic condition characterized by masses of gold-staining material closely associated with the degenerating tree of innervation. These masses were located both externally and internally to the muscle fibers (Figs. 12 to 16). They varied in length from 5 to 105 μ. They were found in 8 of the 23 monkeys studied from 1 to 4 days after the onset of paralysis. They were not found after the seventh day of paralysis. The gold-staining masses were found in the following muscles: biceps brachii, intercostals, quadriceps extensor femoris, biceps femoris, gastrocnemius, tibialis anterior, sternocleidomastoid and trapezius. These gold-staining inclusion masses were composed of granules 0.1 to 2 μ and globoid bodies 2 to 4.5 μ in diameter (Figs. 18 to 26). Each of these bodies was frequently surrounded by a clear halo-like space (Fig. 26). In some places these masses were uniformly and densely stained. In others they had a clear-cut arrangement into cross striations, whereas in still others the periphery of the auriphilous masses was indented in a festoon-like manner by the normal cross striations of the muscle fiber. In many places the granules of the gold-staining masses were aligned in a manner corresponding to that of the normal cross striations of the muscle fiber. In other locations the cross striations of the inclusion masses had a periodicity of their own which did not correspond to that of the immediately related cross striations in the muscle fiber.

It is assumed that these gold-staining inclusion masses were projected from the termination of the degenerating axon into the muscle substance. The assumed relationship of these masses to the axonic substance is based upon two histologic facts; namely, (1) the similarity of the staining capacity of the inclusion masses to that of the axon, and (2) the appearance of the inclusion masses out in the muscle substance corresponding in time to the attenuation of the axonic terminals and diminution of their content of gold-staining substance. In other words, these inclusion masses were assumed to represent an abnormal secretion of the substance of the motor end-plate and a failure of normal dispersal of this product of axonic secretion throughout the muscle fiber.

These inclusion masses had a more intense staining capacity for gold than that of the normal cross striations in the muscle fiber. Since Hurst [13] claimed that he found sporidiosis in some of the muscle fibers

of the monkey, care was taken to differentiate histologically these gold-staining inclusion masses from sporidia. In 18,246 muscle fibers of the left quadriceps extensor femoris muscle (monkey 490, Kramer's series) these inclusion masses were found in 14,164 fibers in close association with the zone of rapidly disintegrating innervation. These inclusion masses had not been found in 30 muscles of 2 normal control monkeys, nor in those examined more than 1 week after the onset of paralysis. These ephemeral inclusion masses at the degenerating myoneural junction appeared to be characteristic, as far as this limited evidence goes, of certain unknown chemical reactions that occur at certain times during the early stages of experimental poliomyelitis within and near the degenerating innervation of muscles in some monkeys. There may be an affinity of the virus of poliomyelitis or its products for acetylcholine or some related substances delivered by the end-plates into the muscle fiber.

The degenerative process resulting in either the diminution or complete depletion of gold-staining substance from within the terminal axons of motor nerves appeared to begin at the end-plates in the muscle fiber and then progressed in a centripetal direction (Figs. 27 to 33). There was first a dissolution of the hypolemmal axons and a related variable quantity of the granules of Kühne that constitute the end-plates. At the beginning the epilemmal axons were engorged, in many places, with gold-staining substance (Figs. 6 and 11) but denuded of end-plates. Within 48 to 96 hours some of the intramuscular nerve trees showed their epilemmal axons with decreased amounts or completely depleted of axonic gold-staining substance. The ghost-like outline of some of the axons had either scattered granules of the fragmented axons or complete absence of granules peripherad, whereas more centrad (Figs. 27 to 33) the same epilemmal axons had a strong affinity for gold because of the persistence of the axonic substance.

There is no adequate chemical explanation as yet for either the morphologic dissolution of the end-plates or the centripetal direction of depletion of the peripheral terminals of the epilemmal axons of their gold-staining substance produced by poliomyelitis. Many experiments were suggested by these findings and more facts must be established before an adequate theory may be advanced to explain these observed facts of the pathology of the motor end-plates.

Passive hyperemia of the capillaries was prominent in some muscles during the pre-paralytic and early paralytic stages of muscle weakness. Each muscle fiber was clearly delimited because of the greatly congested capillaries, the content of which had abnormally increased af-

finity for gold (Figs. 3, 4, 7, 8, 9, and 17). In some places there was a beginning proliferation of the perivascular reticulo-endothelial mesenchymal cells in the weakened muscles. Endothelial vascular sprouts of the beginning of vasculogenesis were observed in some places (Fig. 9).

Summary

1. The early histologic changes that occurred in the neuromuscular mechanism of experimental poliomyelitis in monkeys were studied by the gold method in comparison with the muscles from 2 normal control animals. A quantitative analysis of the anatomic changes was aided by both low-power and high-power photomicrography.

2. The earliest pathologic change was hyperemia and beginning perivascular infiltration of certain areas of the intramuscular blood vessels, which had more granules with strong affinity for gold in the lumen than did the normal. This hyperemia was detected in weakened but nonparalyzed muscles in certain places prior to alterations in the structure of the motor end-plates.

3. On the first day of paralysis some of the end-plates were retracted in ball-like masses and others were hypertrophied and granular. About 20 per cent of the motor plates were absent in the paralyzed muscle on the first day, and within 2 to 4 days about 50 per cent of the end-plates had disappeared. The rates of denervation and degeneration were not only unequal in different muscles of the same animal but in different groups of muscle fibers within the same muscle.

4. Projected masses of gold-staining axonic substance were found in close relationship to the degenerating tree of innervation from 1 to 4 days after the onset of paralysis. They were absent after 7 days of paralysis. They were found in certain muscles of 8 of the 23 monkeys studied. These auriphilous masses were composed of granules and spheroid bodies that varied from 0.1 to 4.5 μ in diameter and in some places were arranged in cross striations which had a more intense affinity for gold than the ordinary cross striations of the muscle fiber. These ephemeral gold-staining masses were not found in 30 muscles of 2 normal control monkeys nor in muscles after 1 week of paralysis. These projected masses of axonic substance at the degenerating neuromuscular mechanism appeared to be characteristic, as far as this limited evidence goes, of certain unknown chemical reactions that occur at certain times during the early stages of experimental poliomyelitis within and near the degenerating innervation of some muscles in some monkeys.

5. The degeneration appeared to begin in the motor end-plates and

then to extend in a centripetal direction through the epilemmal axons in many of the nerve trees observed.

6. The tentative hypothesis is advanced that the histopathologic changes observed at the neuromuscular mechanism during the early stages of poliomyelitis are the result of an abnormal excitation of the secretory mechanism of the motor end-plates which results in the progressive exhaustion of the gold-staining axonic substance leading to denervation at the myoneural junction. This early material exhaustion of the terminals of motor nerves in skeletal muscle by the virus of poliomyelitis will be studied in relation to the chemical secretion of acetylcholine. This experimental study will be extended to include a correlation of the changes in the neuromuscular mechanism with those in the spinal cord and medulla oblongata in the same animal.

I wish to express gratitude to Mr. Leo Massopust, Director of the Department of Art and Photography, for aid with the photomicrographs; to Dr. G. Kasten Tallmadge, Assistant Professor of Anatomy, for reading the manuscript; and to Messrs. Eugene Haushalter, John Schmitz, James Keyes, Joseph Hamel and Robert Jeub for technical aid in the teasing of muscle and nerve plates.

Addendum

Subsequent to the acceptance of this paper for publication evidence has been obtained which supports the statement that metabolic substances such as lactic and other acids may accumulate abnormally during anoxia locally produced in the muscle and thereby destroy the motor end-plates. The abnormal accumulations of ephemeral axonic substances extrinsic to the end-plates undergoing liquefaction are observed in some of the muscle fibers. This is due either to an abnormal permeability of the end-plates and secretion of axonic material or a delay in its dissolution after this material is secreted from nerve to muscle.[19]

It has been established that abnormal amounts of lactic acid appear in the blood in hemorrhagic,[20] traumatic,[21] and "gravity" [22] shock. The pathology of the end-plates leading to their destruction and the evanescent appearance of axonic nervous material in the muscle during the incipient stages of hemorrhagic shock [23] is quite comparable to that of the early effect of lactic acid and poliomyelitis. Gesell and his colleagues found that lactic acid caused a delay in the destruction of physiologically deposited acetylcholine at the synapses.[24, 25] Whether or not the abnormal amount of nervous material histologically observed in muscle in poliomyelitis, in the lactic acid series of experiments, and in experimental shock are the morphological expression of

the temporary appearance of, and the delay in, the dissolution of acetylcholine in muscle cannot be answered conclusively at the present time.

REFERENCES

1. Carey, E. J. Morphologic effects of poliomyelitis virus upon motor end plates in the monkey. *Proc. Soc. Exper. Biol. & Med.*, 1943, **53**, 3–5.
2. Landsteiner, K., and Levaditi, C. La transmission de la paralysie infantile aux singes. *Compt. rend. Soc. de biol.*, 1909, **67**, 592–594.
3. Flexner, S., and Lewis, P. A. Experimental epidemic poliomyelitis in monkeys. *J. Exper. Med.*, 1910, **12**, 227–255.
4. Taylor, H. D. Blood counts in experimental poliomyelitis in the monkey. *J. Exper. Med.*, 1919, **29**, 97–114.
5. Penfield, W. A method of staining oligodendroglia and microglia (combined method). *Am. J. Path.*, 1928, **4**, 153–157.
6. Fairbrother, R. W., and Hurst, E. W. The pathogenesis of, and propagation of the virus in, experimental poliomyelitis. *J. Path. & Bact.*, 1930, **33**, 17–45.
7. Hurst, E. W. A further contribution to the pathogenesis of experimental poliomyelitis: inoculation into the sciatic nerve. *J. Path. & Bact.*, 1930, **33**, 1133–1143.
8. Covell, W. P. Nuclear changes of nerve cells in acute poliomyelitis. *Proc. Soc. Exper. Biol. & Med.*, 1929–30, **27**, 927–929.
9. Toomey, J. A., and Takacs, W. S. Further experiments with poliomyelitis virus in cotton rats. *Proc. Soc. Exper. Biol. & Med.*, 1941, **46**, 319–321.
10. Sabin, A.B., and Olitsky, P. K. The olfactory bulbs in experimental poliomyelitis. *J. A. M. A.*, 1937, **108**, 21–24.
11. Goodpasture, E. W. The Pathology and Pathogenesis of Poliomyelitis. In: Infantile Paralysis. A Symposium Delivered at Vanderbilt University, April, 1941. National Foundation for Infantile Paralysis, Inc., New York, 1941, pp. 85–125.
12. Howe, H. A., and Bodian, D. Neural Mechanisms in Poliomyelitis. The Commonwealth Fund, London, 1942, pp. 4 and 78.
13. Hurst, E. W. The histology of experimental poliomyelitis. *J. Path. & Bact.*, 1929, **32**, 457–477.
14. Hassin, G. B. Landry's paralysis: its clinical and pathologic features. *J. Neuropath. & Exper. Neurol.*, 1943, **2**, 293–300.
15. Wilkinson, H. J. The innervation of striated muscle. *M. J. Australia*, 1929, **2**, 768–793. Experimental studies on the innervation of striated muscle. *J. Comp. Neurol.*, 1930, **51**, 129–151.
16. Carey, E. J. Studies on ameboid motion of motor nerve plates. II. Pathologic effects of CO_2 and electricity on the explosive ameboid motion in motor nerve plates in intercostal muscle. *Am. J. Path.*, 1942, **18**, 237–289.
17. Zenker, F. A. Ueber die Veränderungen der willkührlichen Muskeln im Typhus abdominalis. A. E. Junge, Erlangen, 1863; F. C. W. Vogel, Leipzig, 1864.
18. Wells, H. G. The pathogenesis of waxy degeneration of striated muscles (Zenker's degeneration). *J. Exper. Med.*, 1909, **11**, 1–9.

REFERENCES FOR ADDENDUM

19. Carey, E. J., and Massopust, L. Sudden destruction of motor end plates by lactic acid. *Proc. Soc. Exper. Biol. & Med.*, 1944, **55**, 194–197.

20. Govier, W. M., and Greer, C. M. The effect of certain anesthetics on blood keto acid levels. *J. Pharmacol. & Exper. Therap.*, 1941, **73**, 441–445.

21. Gutmann, H., Olson, W. H., Kroll, H. H., Levinson, S. O., and Necheles, H. Chemical studies in traumatic shock. *Am. J. Physiol.*, 1941, **133**, 308–309.

22. Cole, W. H., Allison, J. B., Leathem, J. H., Nastuk, W. L., and Anderson, J. A. Acidosis in the rabbit during "gravity shock." *Anat. Rec.*, 1942, **84**, 468.

23. Carey, E. J., Massopust, L. C., Zeit, W., Haushalter, E., and Schmitz, J. Acute anatomic breakdown of motor end plates in hemorrhagic shock. *Proc. Soc. Exper. Biol. & Med.*, 1944, **56**, 115–118.

24. Gesell, R., Brassfield, C. R., and Hansen, E. T. Possible rôles of cH in neurophysiology. *Proc. Fed. Am. Socs. Exper. Biol.*, 1942, **1**, 29.

25. Hansen, E. T., Worzniak, J. J., and Gesell, R. Physiological effects of artificially administered acetylcholine and eserine. *Proc. Fed. Am. Socs. Exper. Biol.*, 1942, **1**, 36.

[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 172

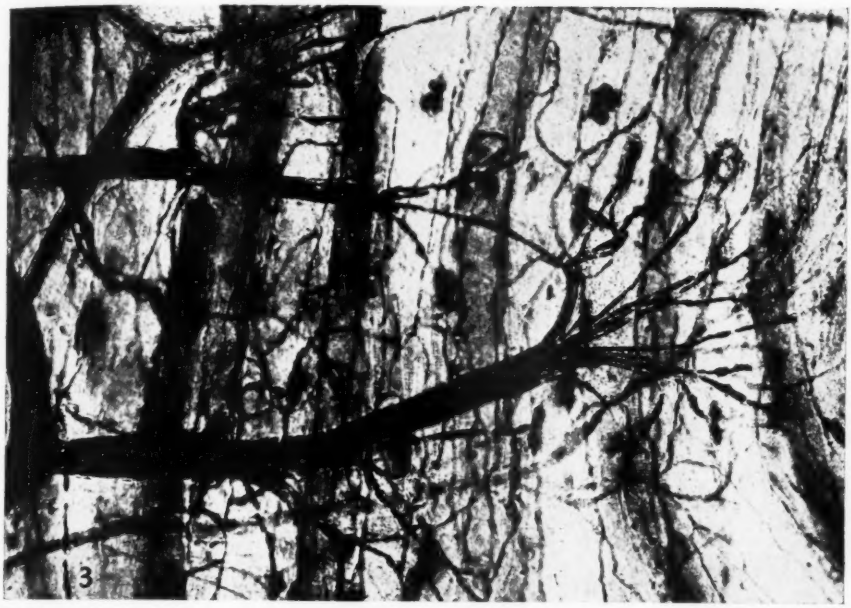
FIGS. 1 and 2. The teased normal innervation of the left quadriceps femoris muscle (Fig. 1) in the monkey is contrasted with that in the left quadriceps femoris muscle (Fig. 2) on the first day of paralysis. In the normal (Fig. 1) there is a variation in the size of the motor end-plates and there are present muscle fibers that are darkly and lightly stained with gold. On the first day of paralysis (Fig. 2) some of the motor end-plates, which stain deeply with gold, are uniformly retracted, and there is a loss of the differential types of muscle fibers. There is passive hyperemia of the capillaries of the muscle fibers (Fig. 2). × 150.

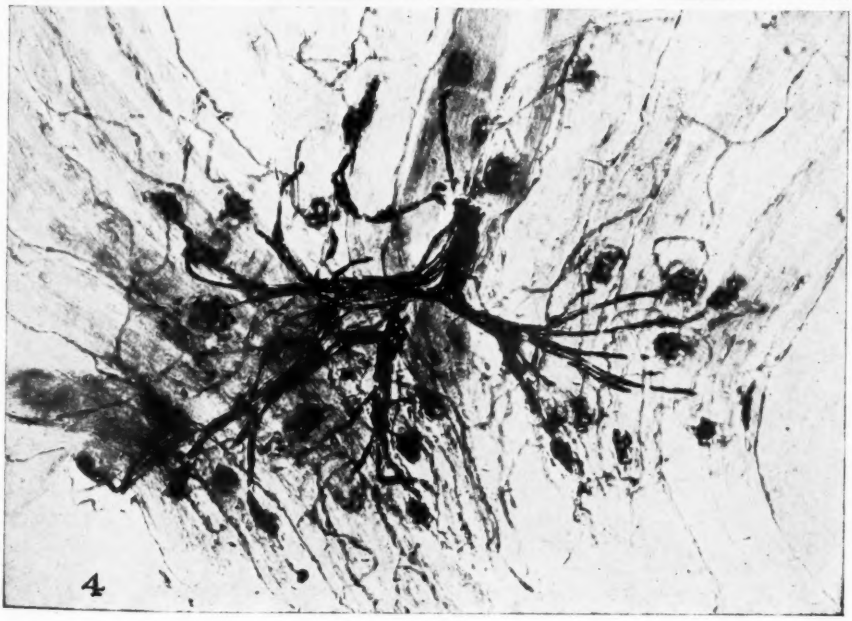
Carey

Effects of Poliomyelitis on Neuromuscular Mechanism

PLATE 173

FIGS. 3 and 4. The teased innervation of the nonparalyzed but weakened gastrocnemius muscle from a monkey in which the upper extremities were paralyzed for 1 day. There is definite enlargement and hyperemia of the intramuscular blood vessels. The motor end-plates are in variable degrees of retraction, and some are in a state of granular fragmentation. The epilemmal axons are beaded and in some places fragmented. × 150.



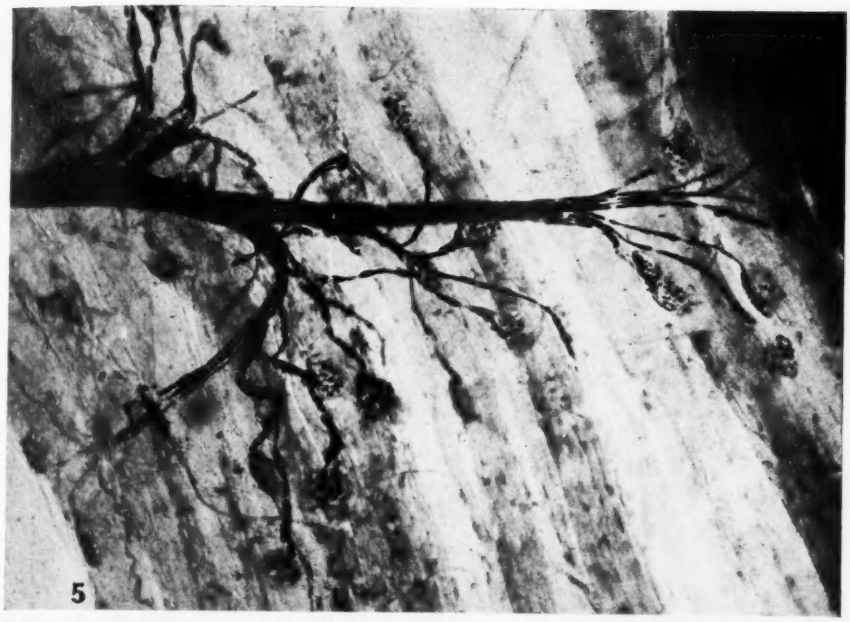


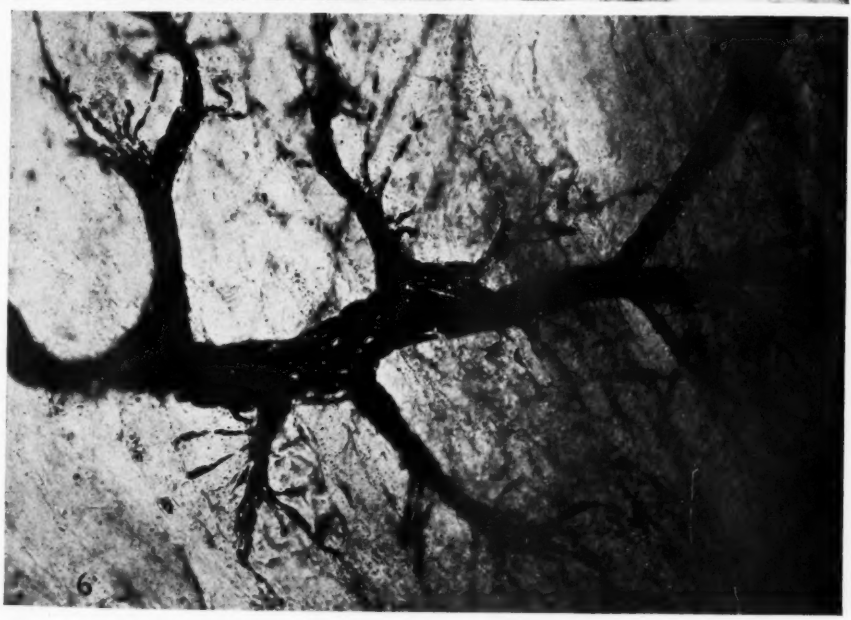
Carey

Effects of Poliomyelitis on Neuromuscular Mechanism

PLATE 174

FIGS. 5 and 6. The teased innervation of the nonparalyzed but greatly weakened triceps muscle (Fig. 5) in contrast to the paralyzed biceps brachii muscle (Fig. 6) from the same monkey (no. 490, Kramer's series). The hypolemmal axons of the motor end-plates are fragmented into granules which have a stippled pattern and take a deep stain with gold (Fig. 5). The epilemmal axons are irregularly beaded. The motor end-plates have completely disappeared in the biceps brachii muscle (Fig. 6) except in a few locations where a diffuse granulation is found. The epilemmal axons are definitely enlarged, shortened and beaded, and terminate in rounded or ovoid bulbs which are enlarged and are stained deeply with gold. In many places the cross striations of the muscle fiber have disappeared and a diffuse granulation is present. × 150.





Carey

Effects of Poliomyelitis on Neuromuscular Mechanism

PLATE 175

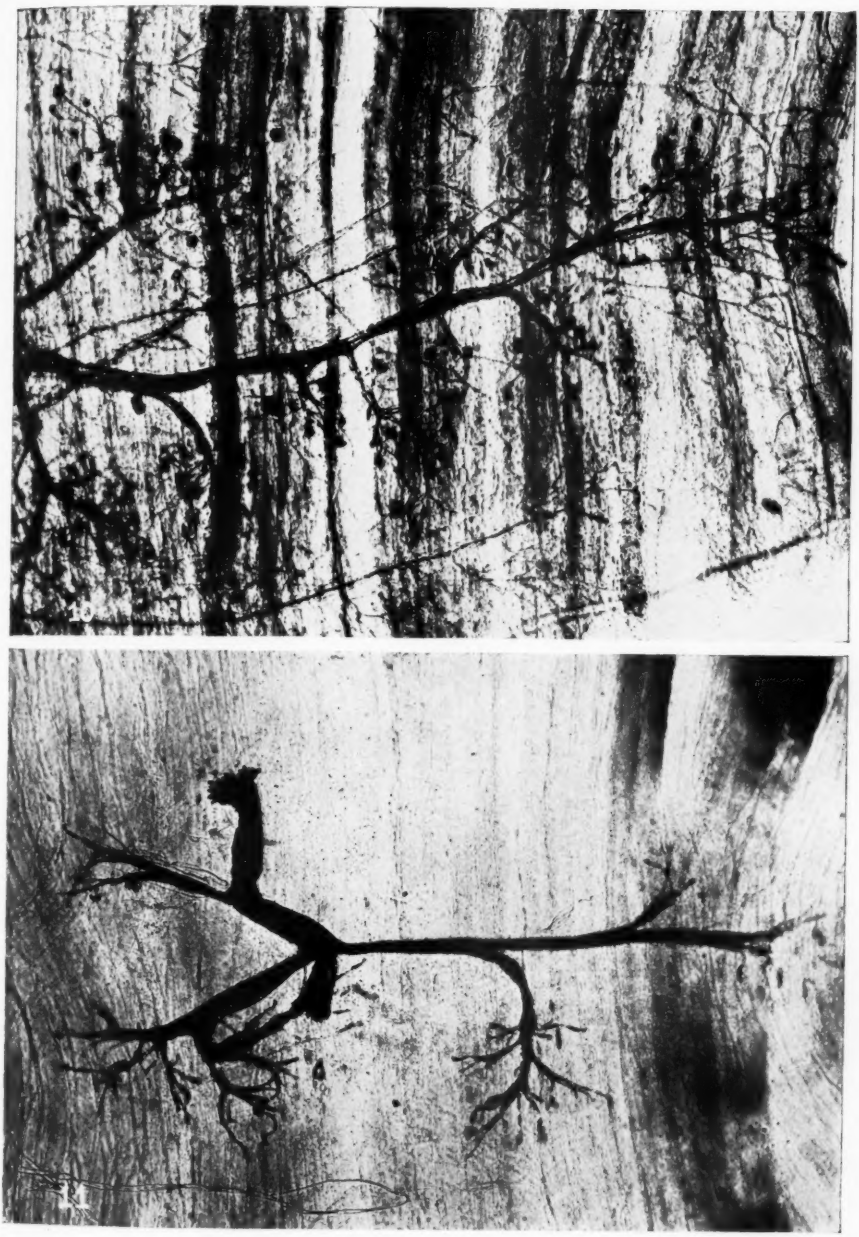
FIGS. 7, 8 and 9. The teased motor innervation from the nonparalyzed but weakened biceps femoris muscle (Figs. 7, 8 and 9) 1 day after the onset of the paralysis of both upper extremities. There was muscle weakness associated with a striking passive hyperemia and enlargement of the capillaries and the intramuscular vessels, with beginning perivascular infiltration of leukocytes and a few lymphocytes. There is increased visibility in the nuclei of some muscle fibers (Fig. 8) arranged in longitudinal rows. A majority of the motor endplates show retraction and increased staining capacity. There is beading of the epilemmal axons. The content of the congested blood vessels has increased staining affinity for gold. × 80.

Carey

Effects of Poliomyelitis on Neuromuscular Mechanism

PLATE 176

FIGS. 10 and 11. Teased innervation of the nonparalyzed but weakened biceps brachii muscle (Fig. 10) of the monkey. There is definite granular degeneration of some muscle fibers and passive hyperemia of the intramuscular capillaries. The epilemmal axons are shortened and beaded, and the hypolemmal axon of the end-plate is retracted into rounded, deep-staining terminals. The motor innervation of the paralyzed triceps muscle (Fig. 11) is from the opposite limb to that illustrated above (Fig. 10). There is a loss of the majority of the motor end-plates and the faint outline of those present is occupied by granules diffusely arranged. The epilemmal axons are retracted, beaded, enlarged, and have intense affinity for gold. × 80.



Carey

Effects of Poliomyelitis on Neuromuscular Mechanism

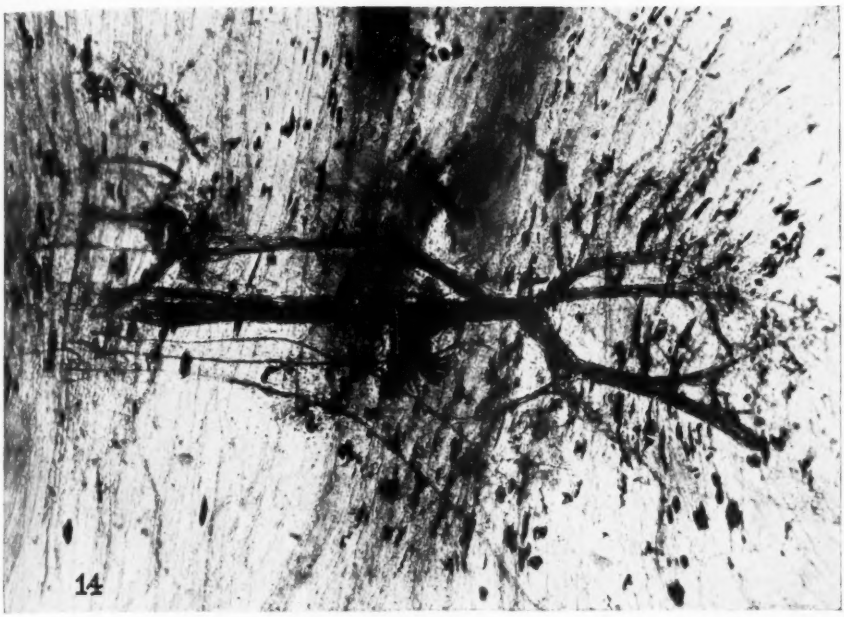
PLATE 177

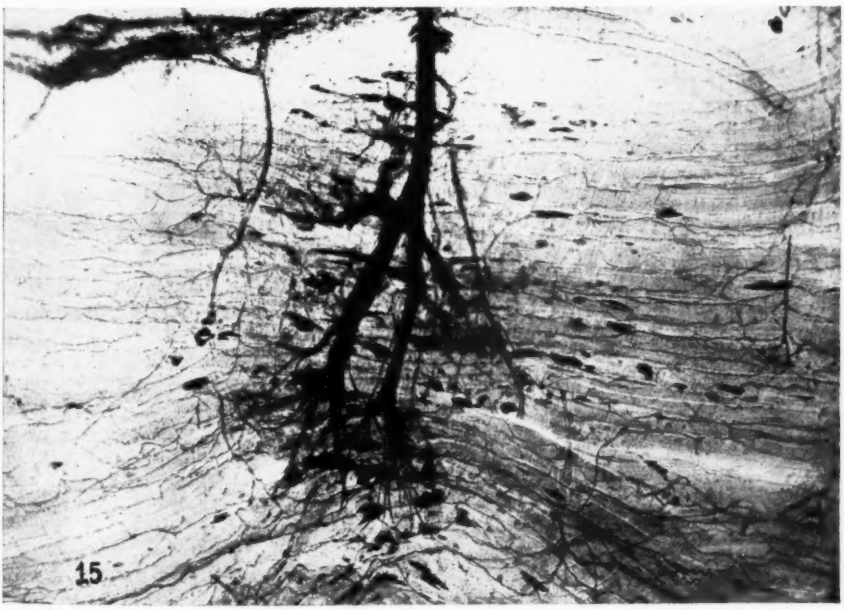
FIGS. 12 and 13. The motor innervation of the paralyzed right biceps brachii muscle (Fig. 12) of monkey 490, Kramer's series. There is a loss of motor end-plates but there are present multiple masses, both inside and outside of the muscle fiber, that have the same intense affinity for gold as that of the normal axon. It is assumed that these masses were projected from the termination of the axon into the muscle substance. The relationship of these masses to the axonic substance is based upon two factors; namely, (1) similarity of staining capacity and (2) the attenuation of the terminals of the axon simultaneously with the presence of the gold-staining masses in close relationship to the motor innervation. The motor innervation of the left paralyzed biceps brachii muscle (Fig. 13) in the same monkey likewise has these gold-staining inclusion masses in close relationship to the attenuated terminals of the axons. There are endothelial sprouts of new blood vessels in a state of passive hyperemia (Fig. 13). × 80.

Carey Effects of Poliomyelitis on Neuromuscular Mechanism

PLATE 178

FIGS. 14 and 15. The teased motor innervation of the paralyzed right gastrocnemius muscle (Fig. 14) of monkey 338, Pearson's series. There is a close relationship of the projected gold-staining masses to the attenuated terminals of the axons. These inclusion masses vary in size from rounded, globoid bodies, 1 to 2 μ in diameter, to masses of these granules that vary from 5 to 105 μ in length. The teased motor innervation of the paralyzed left tibialis anterior muscle (Fig. 15) in the same animal as above (Fig. 14) not only possesses retracted deep-staining motor end-plates but closely related gold-staining inclusion masses which appear simultaneously with the attenuation and, in some instances, with complete depletion of the gold-staining substance of the axons. $\times$ 80.



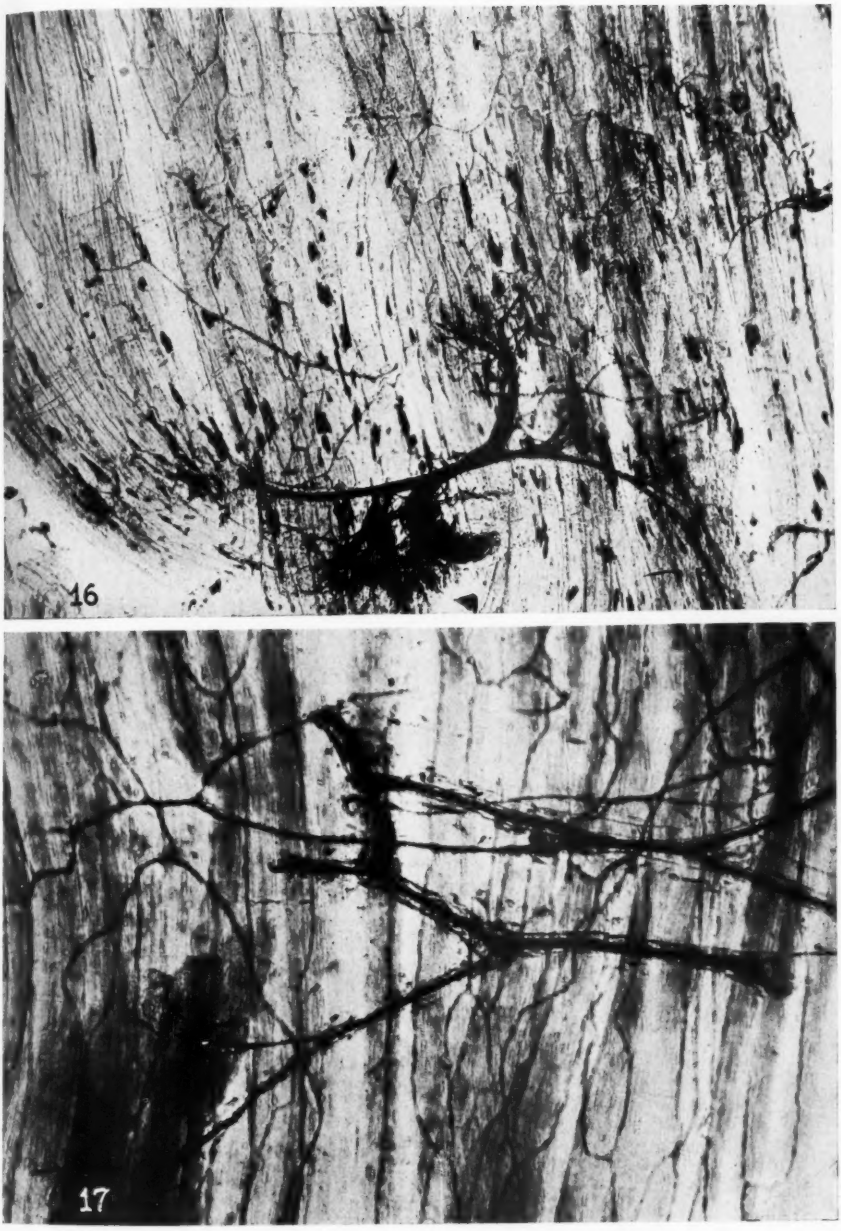


Carey Effects of Poliomyelitis on Neuromuscular Mechanism

PLATE 179

FIG. 16. The teased motor innervation of the right sternocleidomastoid muscle of monkey 53, Marquette series, had projected gold-staining masses which varied from 5 to 105 μ in length and which were related to the degenerating innervation. The absence of the motor end-plates concomitant with the appearance of the gold-staining projection masses in the muscle fiber is clearly evident. $\times$ 80.

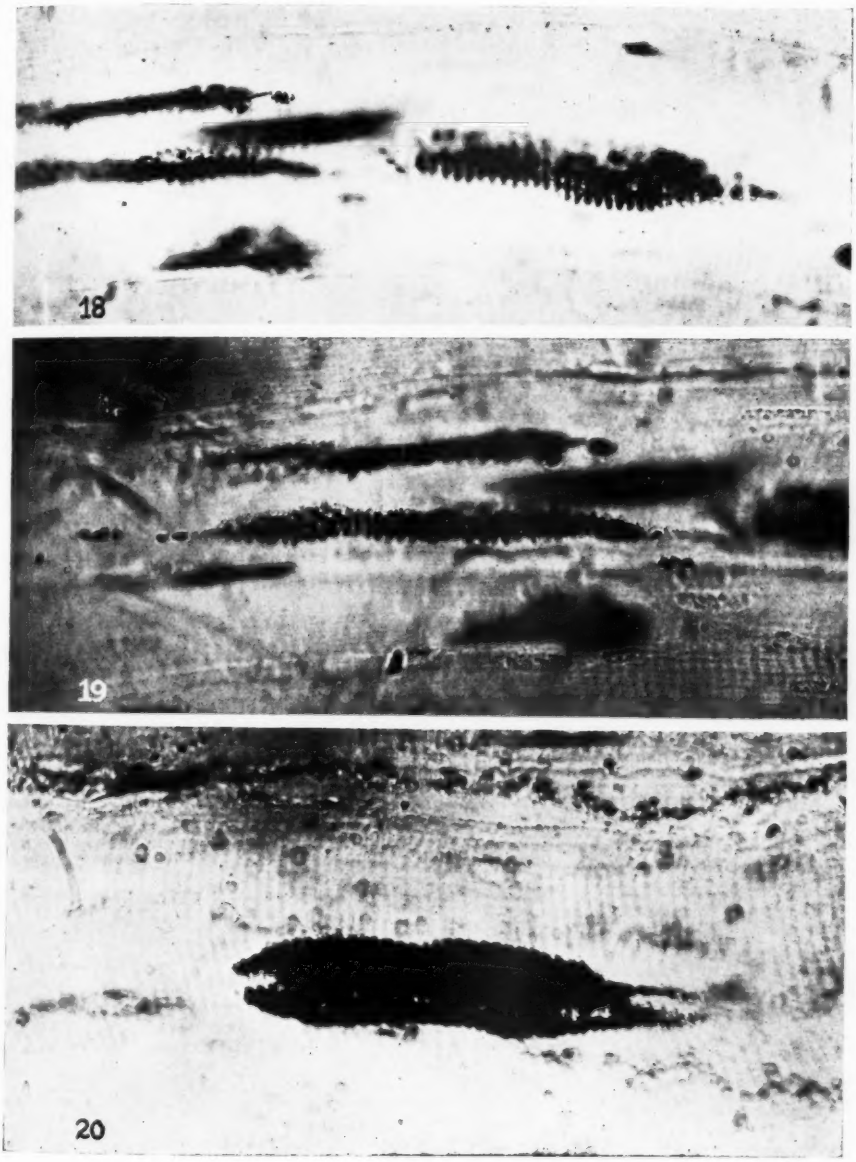
FIG. 17. The teased fibers of the weakened right trapezius muscle of monkey 53 had enlarged and congested intramuscular blood vessels. There is evidence of a beginning perivascular infiltration of polymorphonuclear leukocytes and some lymphocytes. Passive hyperemia with enlargement of the blood vessels is one of the earliest signs of pathologic change in the involved muscle. $\times$ 150.



Carey Effects of Poliomyelitis on Neuromuscular Mechanism

PLATE 180

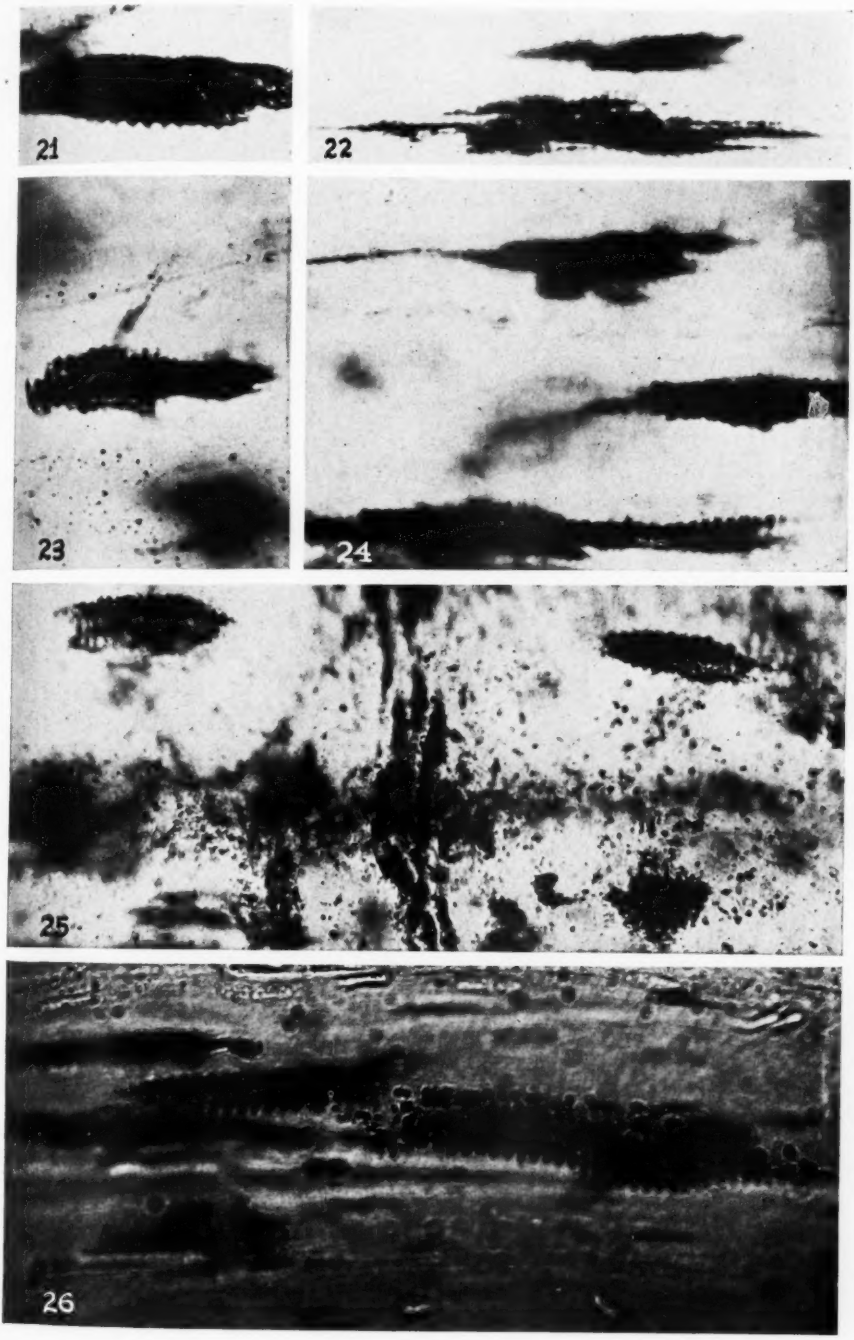
FIG. 18, 19 and 20. Elongated projection masses of axonic substance in the paralyzed gastrocnemius muscle (Figs. 16, 17 and 18). These projection masses have a greater affinity for gold than the dark cross striations of the muscle fiber. These masses are composed of rounded bodies 1 to 4 μ in diameter, and in some places are arranged in cross striations. In other places the periphery has a festooned border, the convexities of which correspond with the ordinary dark cross striations of the muscle fiber. (Fig. 18). $\times$ 750.



Carey Effects of Poliomyelitis on Neuromuscular Mechanism

PLATE 181

FIGS. 21 to 26. Elongated projection masses of axonic substance in the paralyzed gastrocnemius muscle 2 days after the onset of paralysis. Some of these projection masses have a deep uniform stain, the border of which is festooned (Fig. 21). In others there is a definite arrangement of the globoid granules into cross striations. Some of these globoid granules arranged in cross striations are surrounded by a light circular border (Fig. 26). In some places these granules are aligned to correspond with the normal cross striations of the muscle. In other locations they have a periodicity of their own which does not correspond to the immediately related cross striations in the muscle fiber (Fig. 26). These axonic projection masses have a more intense affinity for gold than that of the cross striations of the muscle. In some places (Fig. 25) there is a diffuse arrangement of the projected axonic granules in relationship to others which are definitely cross striated. × 750.

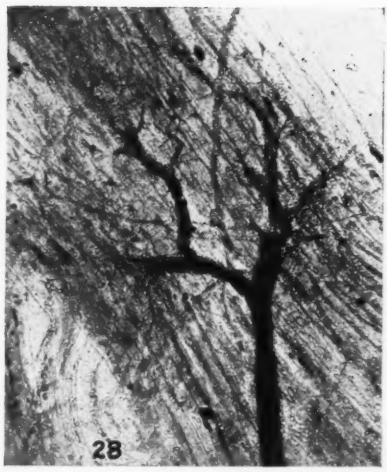


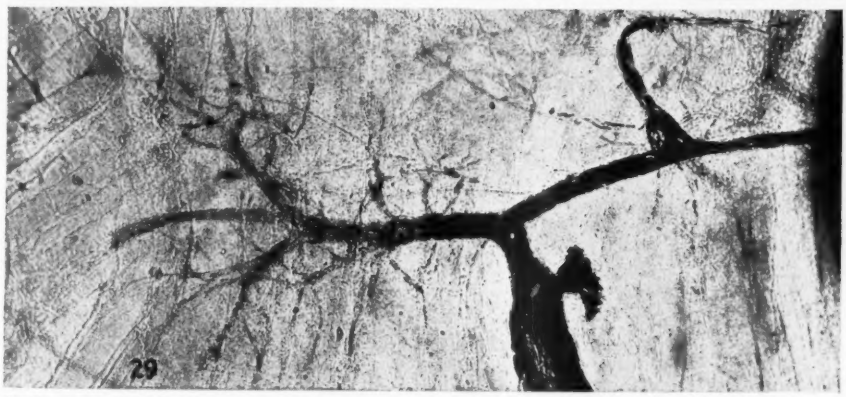
Carey

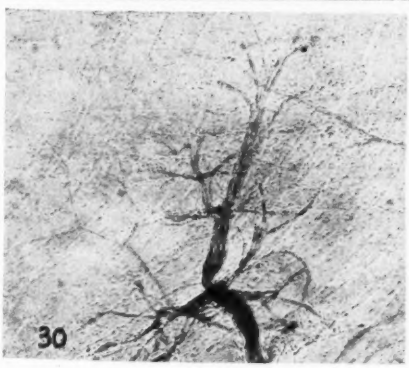
Effects of Poliomyelitis on Neuromuscular Mechanism

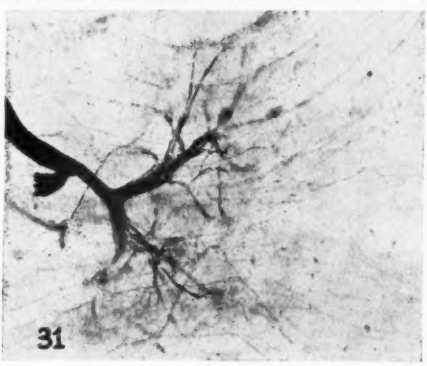
PLATE 182

FIGS. 27 to 31. Elongated projection masses of axonic substance in the paralyzed biceps brachii muscle. It may be noted that corresponding with the presence of the axonic masses projected into the substance of the muscle fiber there is a decrease of gold-staining material in the axons, 4 days after the onset of paralysis (Figs 27 and 28). There is an absence of gold-staining axonic masses in the paralyzed triceps muscle (Figs. 29, 30 and 31), 4 days after the onset of paralysis, taken from the same limb (Figs. 27 and 28). There is a diminution or complete depletion of the substance in the axonic terminals. This pathologic change appears to progress in a centripetal direction from the original location of the motor end-plates. The terminal epilemmal axons in some places (Figs. 29, 30 and 31) have merely a faint ghost-like outline with no affinity for gold, or a greatly decreased staining capacity for gold. × 80.









Carey

Effects of Poliomyelitis on Neuromuscular Mechanism

PLATE 183

FIGS. 32 and 33. There is a decrease, or a complete exhaustion, of the gold-staining substance in the terminal axons of the motor innervation of the paralyzed triceps muscle, 4 days after the onset of paralysis. This pathologic change appears to progress in a centripetal direction from the original location of the motor end-plates. The terminal epilemmal axons in some places (Figs. 32 and 33) have merely a faint ghost-like outline with no affinity for gold or a greatly decreased staining capacity for gold. Corresponding to the complete depletion of the substance in the terminal axons there is an eventual disappearance of the axonic inclusion masses that were originally projected into the substance of the muscle fiber. In some places, the cross striations of the muscle fibers are replaced by a disorganized mass of granules and in others by a more hyaline appearance comparable to Zenker's waxy degeneration. × 250.

Carey

Effects of Poliomyelitis on Neuromuscular Mechanism

CONGENITAL GASTRO-ENTERIC CYSTS OF THE MEDIASTINUM

A Review and Report of a Case *

Harry G. Olken, M.D.
(*From the Department of Pathology, Tufts College Medical School, Boston, Mass.*)

Tumors of the mediastinum, as a group, are not common, and of these only a minor percentage are cystic.[1] Cysts of the mediastinum are usually congenital and represent for the most part dermoid or teratoid tumors. Other cysts have been described in association with the various structures of the mediastinum. The simplest of these are represented by the pericardial celomic cysts [2] and the cystic lymphangiomas.[3] The so-called bronchiogenic cysts, although somewhat more common, are also more complex.[4] The latter are lined with ciliated cylindrical epithelium, contain mucous glands, muscle and cartilage, and are referred to misplaced portions of the respiratory tract. Still another group of cysts are lined by mucous membrane resembling some portion of the intestinal tract and are classed as enteric or gastric cysts.[5]

The following case falls into this latter category of gastro-enteric cysts of the mediastinum, of which fewer than 20 have hitherto been reported in the literature.

Report of Case

(Tufts Medical School, Pathology no. A-41-2.) A full-term, male, stillborn fetus weighing 7 lbs. was delivered at the Evangeline Booth Maternity Hospital. The mother was a healthy white primipara. The child showed hydrocephalus and spina bifida on external examination. Autopsy revealed additional findings involving not only the central nervous system but also the thoracic cavity.

The head was enlarged and the suture lines widely separated. Over the lower thoracic and upper lumbar area of the back there was a large cutaneous defect (4.0 by 2.5 cm.), associated with an underlying defect in the spinal column, in which the arches had failed to close. From the cervical region to the sacrum there was only one completely fused arch —the first thoracic. There was, furthermore, herniation through the foramen magnum of the elongated cerebellum, which extended down the vertebral canal, overlying the spinal cord. The tonsils of the cerebellum lay at the level of the first thoracic vertebra. At the mid-thoracic region the spinal cord split into two flattened ribbons which were incorporated in a meningocele. The cranial bones showed numerous, irregular fenestrations. The dura was completely absent within the cranial cavity. The gyri of both hemispheres were small.

* Received for publication, December 24, 1943.

Exposure of the thoracic cavity showed a small, bilobed, right lung, which was compressed by a large cystic mass extending from the posterior mediastinum and occupying the greater portion of the right thorax. The mass lay behind the pleura, and showed no communication with the trachea, esophagus, or any other mediastinal structure (Fig. 1). The heart and the left lung were pushed to the left. The cyst extended from the right apex to the diaphragm and measured 8.0 cm. in greatest length by 4.0 cm. in greatest diameter. It contained thick, clear, mucoid fluid. Its wall varied in thickness from 0.2 to 0.4 cm., and its inner surface was smooth. Sections were removed for histologic examination. The organs of the abdomen showed no anomalies.

Anatomic Diagnoses. (Gastrogenic) cyst of the posterior mediastinum.

Spina bifida with diplomyelia and myelomeningocele; Arnold-Chiari malformation [6] of the cerebellum (herniation of the cerebellum into the spinal canal); microgyria; congenital absence of the dura, including tentorium, falx, and dural sinuses; congenital fenestration of the cranial bones (craniolacunia). Hydrocephalus. Bilobed right lung.

Microscopic Examination of the Wall of the Mediastinal Cyst

The wall of the mediastinal cyst varied in thickness and in structure. In areas it was thin and composed of a few muscle fibers and loose connective tissue. In other areas distinct muscle layers (circular, transverse and oblique) were noted. Varying amounts of lymphoid tissue were seen in different areas of the wall. The cyst was lined with mucosa, although in places it was denuded. The mucosa varied from a simple layer of cuboidal, nonciliated epithelium, a single cell in thickness, to a well differentiated gastric lining in which mucus-secreting glands and acid-secreting cells were identified (Figs. 2, 3 and 4).

Review of the Literature

An intensive search revealed 18 well defined cases of gastro-enteric cysts of the mediastinum. In the following table these cases are summarized in brief.

Although so small a series does not lend itself to statistical analysis, many interesting features appear to be common to the group. Somewhat more common in males than in females, these cysts, for the most part, occupy the right hemithorax, although occasionally they are found on the left side or in a median position. Most striking is the early age at which symptoms are produced. Seventy-five per cent of the cases were discovered within the first year of life and in only two instances [19, 22] were the children as old as 3 years. This is in sharp

Summary of Reported Cases

No.	Author (year)	Sex and age	Description of mediastinal cyst	Other anatomic findings, and remarks
1	Hennig[7] (1880)	F Newborn	Extended into left pleural cavity, from 3rd to 5th vertebra; size of plum; lined with simple cylindrical epithelium	Intramesenteric cyst lined with similar epithelium
2	Roth[8] (1881)	M Newborn	Extended into right pleural cavity; lined with "typical intestinal mucosa"	Two enteric cysts of abdomen lined with similar epithelium, plus a diverticulum of ileum extending between the layers of mesentery
3	Staehelin-Burckhardt[9] (1909)	F 9 mos.	At lower end of esophagus on right side; size of hen's egg; wall showed structure of both stomach and esophagus plus areas of ciliated epithelium	Spina bifida and uvula bifida; diverticulum of urinary bladder
4	Schmincke[10] (1920)	M 15 da.	Extended into right thoracic cavity; size: 4.5 by 4.3 by 4.0 cm.; lined with small intestinal mucosa	Intramesenteric enteric cyst; congenital scoliosis; extra lobe of right lung
5	Mixter and Clifford[11] (1929)	M 22 mos.	Filled two-thirds of right thorax, extending from apex to diaphragm; 6.6 cm. in average diameter; lined with gastric mucosa	No other anomalies noted; cyst removed surgically, and child recovered
6	Mixter and Clifford[11] (1929)	M 7 wks.	Bilocular cyst lay for the most part in the right thorax; 5.0 cm. in greatest diameter; lined with typical gastric mucosa	No other anomalies noted; removal attempted, but child died
7	Smith[12] (1930)	M 13 mos.	Posterior mediastinal cyst occupied portion of right thorax; size: 12.5 by 3.8 by 3.8 cm.; lined in part by gastric mucosa and in part by ciliated and stratified epithelium	No other anomalies noted at autopsy
8	Fischer[13] (1930)	F 6 mos.	Paravertebral cyst, extended from clavicle to diaphragm behind right lung; size: 8.0 by 4.0 cm.; lining "characteristic of intestines, with areas suggestive of pyloric mucosa"	Scoliosis; accessory spleen
9	Entz and Orosz[14] (1930)	M 11 mos.	Extended into right thorax; size: 10 by 7 by 2.0 cm.; lined with mucosa characteristic of fundal portion of stomach	Scoliosis; mental retardation (clinical); cyst incised and drained; died in 18 days
10	Poncher and Milles[15] (1933)	M 29 mo.	Two cysts of posterior mediastinum extended into right thorax; larger measured 12 by 10 cm.; smaller, 6 by 3 cm.; lined with "typical gastric mucosa"	Intramesenteric diverticulum arising from ileum, containing gastric mucosa with peptic ulcer; died during attempted removal of larger cyst

No.	Author (year)	Sex and age	Description of mediastinal cyst	Other anatomic findings, and remarks
11	Stoeckel[16] (1935)	F Premature, newborn	Size: 5 by 3.5 cm.; straddled the vertebral column at level of 2nd to 6th ribs; constricted in center to form 2 connected cysts; lined with columnar nonciliated epithelium	Abnormal vessel arose from arch of aorta
12	Black and Benjamin[17] (1936)	M 4½ mos.	Retropleural cyst (14.0 by 6.5 cm.) extended into left pleural cavity from 3rd to 12th vertebra; lined with elongated and flattened mucosal cells	Diverticulum of jejunum with a peptic ulcer which had ruptured and produced peritonitis
13	Brass[18] (1936)	F Premature	Kidney-shaped cyst extended into right pleural cavity (7.1 by 3.5 cm.); supplied by intercostal arteries and vagus nerve; lined with "typical small intestinal mucosa"	Uvula bifida
14	Böss[19] (1937)	M 3¾ yrs.	Cyst extended into right thorax; size of hen's egg; lined with mucosa resembling fundal portion of stomach; peptic ulcer of cyst had ruptured in lower lobe of right lung	No other anomalies; died of hemorrhage during second stage of attempted removal
15	Guillery[20] (1937)	Sex ? 3 mos.	Cyst of posterior mediastinum lay on the vertebral bodies from 3rd to 7th thoracic vertebra; size: 5 by 2.5 cm.; second smaller cyst (1.5 by 0.8 cm.) behind 5th thoracic vertebra within spinal canal; both lined with cylindrical epithelium	Distortion of 5th thoracic vertebra
16	Seydl[21] (1938)	F 3½ mos.	In posterior mediastinum, between esophagus and lower lobe of right lung; size: 3.5 by 1.7 by 1.7 cm.; lined with gastric mucosa and contained a peptic ulcer which had perforated into lung and had eroded a large vessel, causing fatal hemorrhage	Left-sided scoliosis; obliterative pleuritis
17	Nicholls[22] (1940)	F 3 yrs.	Right-sided retropleural cyst filled most of right thorax; lined with gastric mucosa	Dorsal scoliosis; cyst incised at age of 3, following which draining sinus developed; completely excised at age of 8, with good healing
18	Carlson[23] (1943)	M 4 mos.	Cyst of posterior mediastinum lined with gastric mucosa	No other anomalies; completely and successfully removed
19	Author	M Newborn	Occupied greater portion of right thorax; size: 8 by 4 by 4 cm.; extended from apex to diaphragm; lined with gastric mucosa with areas of simple cuboidal cylindrical epithelium	Spina bifida; Arnold-Chiari malformation of cerebellum; absence of dura; craniolacunia; hydrocephalus; bilobed right lung

contrast to the mediastinal dermoid tumors which produce practically no symptoms over a period of years while the cyst is undergoing progressive growth and expansion. Of 139 thoracic dermoids and teratomata collected by Kerr and Warfield [24] in 1928, including their own case, only 11 occurred in the first decade of life.

It is a well known axiom in pathology that congenital anomalies are very frequently multiple. This series of cases is no exception, for in almost all instances the thoracic cyst was associated with one or more other anomalies of development. Of the 19 cases analyzed, only 3 failed to show some other developmental anomaly in addition to the mediastinal cyst. The intestinal tract and skeletal system were most frequently affected. In 5 of the 19 cases, single or multiple intra-abdominal enteric cysts, or intestinal diverticula, or both, were found with the gastric or enteric cyst of the thorax. In 8 cases the vertebral column was involved. Usually this involvement was limited to a simple scoliosis, but in some instances the vertebral bodies themselves were misshapen and in 2 instances a severe spina bifida was present.

The majority of the mediastinal cysts were lined, at least partially, by histologically identifiable gastric mucosa (12 of the 19). The mucosal linings of the others were more suggestive of small intestinal epithelium. Böss [19] and Seydl [21] reported active peptic ulcers within the mediastinal cysts and in both instances the ulcers had perforated into neighboring structures. Poncher and Milles [15] and Black and Benjamin [17] also found active peptic ulcers, but in their cases the ulcerations were within the intestinal diverticula which were associated with the mediastinal cysts. This high incidence of peptic ulceration in ectopic gastric mucosa offers an interesting problem for the physiologist and clinician.

The knowledge of these mediastinal anomalies and the improvement in the technics of thoracic surgery have permitted some hope of successful therapy. In at least 7 instances surgical removal of the mediastinal cyst was attempted, and in 3 instances the operation was successful, and recovery complete.

Discussion

Intraperitoneal enteric cysts constitute a rather well defined group of tumors.[25] Appearing as single or multiple cysts of varying size, they resemble in structure the intestines, containing smooth muscle, mucosa, crypts, lymphoid tissue, and a lining of cylindrical, cuboidal, or stratified epithelium. They are usually associated with the small bowel or its mesentery, being situated either at the navel or at the lower end of the ileum. Some, however, have appeared in the cecum [26] or even as

low down as the rectum.[27] These enteric cysts have usually been accepted as originating from Meckel's diverticulum, the omphalomesenteric duct, or from definitely misplaced portions of the intestines.[5]

The appearance of such cysts above the diaphragm has complicated, somewhat, the problem of genesis. Many of the mediastinal cysts which have been described were lined with intestinal epithelium and resembled in all detail the intra-abdominal enteric cysts. An even greater proportion of these mediastinal cysts were lined with gastric mucosa. There is still another group of mediastinal cysts arising from the digestive tract which complicate the picture even further, and should be included in this discussion. These are the esophageal cysts which, because of their position and gross appearance, may be confused with the gastric or enteric cysts of the mediastinum.

Although there can be no doubt that all of these mediastinal cysts are closely related embryologically, the esophageal cysts seem to form a rather distinct group. Several have been described.[28-36] Almost invariably found in adults, they are situated in the lower half of the esophagus, usually between the muscle layers of the esophageal wall. The epithelium is composed of ciliated cuboidal cells with occasional foci of flattened epithelial cells. Histologically these cysts resemble most closely the group described as of bronchiogenic origin,[4] except that the esophageal cysts contain no cartilage.

A discussion of the origin of gastric and enteric mediastinal cysts cannot be dissociated from a consideration of esophageal and bronchiogenic cysts. There is much histologic overlapping. Smith [12] and Staehelin-Burckhardt,[9] for example, reported mediastinal cysts which were lined in part with gastric and in part with ciliated epithelium. Cilia are not restricted to respiratory epithelium. In many regions of the body there occur small or large cysts with ciliated epithelium. In the case of the esophageal cysts, the epithelium is undoubtedly a remnant of the embryonic esophageal epithelium which is ciliated.[37] Also the esophagus and trachea arise in close union. In the 4 mm. pig the lung buds appear attached to the ventral border of the esophagus. The trachea becomes separated from the esophagus by the downward growth of the lung buds and the upward extension of the notch between the lung buds and the esophagus.[38] Mixter and Clifford [11] believed that this phenomenon might also account for the appearance within the mediastinum of gastric cysts. They pointed out that:

". . . The fusion of the lateral walls to form the tracheo-oesophageal septum begins from below. It would seem that at this embryonic stage, the pinching off of an outbud or diverticulum of foregut containing entoderm and mesoderm, and destined to become a portion of the stomach might well occur. This could be carried along by the downward growing lung bud and lodge in the mediastinum or on the surface of the lung."

There is probably more than one explanation for gastro-enteric mediastinal cysts, however. Black and Benjamin,[17] among others, believed that they represent an intrathoracic vestige of the ductus omphalomesentericus (vitelline duct). They repeat the observation made long ago by Fitz [39] that the persistence of the vitelline duct accounts for many cases of intestinal duplication and cyst formation. It is generally accepted that many intra-abdominal variations in intestinal development are due to persistence and growth of vitelline duct remnants. Since the thoracic and abdominal cavities are not differentiated at that period of fetal life when the vitelline duct is present, it is conceivable that this duct might also account for developmental defects within the thorax. In fact, in the case recorded by Black and Benjamin the intrathoracic cyst was associated with a reduplicated intramesenteric intestinal pouch, a defect long attributed to vitelline duct persistence.

Several of the gastric and enteric cysts of the mediastinum were associated with other intra-abdominal enteric cysts or diverticula, some of which were found within the leaves of the mesentery. Poncher and Milles [15] believed that it is difficult to correlate the wide variety of positions of these enterogenous cysts or diverticula with vitelline duct rests. They claimed that:

> ". . . at the time of obliteration of the vitelline duct in the 7 mm. embryo the dorsal mesentery and its vessels are already well developed. In the case of the intramesenteric cysts and diverticula it is necessary to postulate that the duct remnants not only insert themselves between the well formed leaves of the dorsal mesentery, but also between its vessels, deriving an entirely new blood supply from them."

Poncher and Milles were, therefore, more prone to seek another source for these widely separated anomalies, and they found their explanation in the epithelial nodules of Lewis and Thyng.[40] These "epithelial nodules" are definitely misplaced portions of the intestines which can be traced to small diverticula of the gut occurring in fetal life. Such diverticula are apparently not uncommon in human and animal embryos, and occur at a time when their persistence would place them between the leaves of the mesentery or within the thoracic cavity.

In summary, then, we find at least three possible explanations for these enteric and gastric cysts. They have been ascribed to the pinching off of a bud or diverticulum of the embryonic foregut; to an intrathoracic remnant of the omphalomesenteric duct; and to an embryonic diverticulum or epithelial remnant capable of producing intestinal or gastric mucosa ("epithelial nodules" of Lewis and Thyng [40]). Probably all three mechanisms play some part in their genesis.

Summary

Congenital gastric and enteric cysts of the mediastinum are rare. A case of a gastric mediastinal cyst is reported and the literature, including 18 previously recorded cases, is reviewed. The origin of such cysts and their relationship to bronchiogenic and ciliated esophageal cysts is discussed.

A study of these 19 cases has failed to reveal an exact reduplication of dysontogenetic patterns, yet it should be pointed out that the common association of mediastinal cysts, mesenteric cysts or diverticula, and deformities of the skeletal system is an interesting finding that is worthy of academic and practical consideration.

REFERENCES

1. Smith, L. W., and Stone, J. S. Tumors of the mediastinum in children. *Ann. Surg.*, 1924, **79**, 687–709.
2. Lambert, A. V. Etiology of thin-walled thoracic cysts. *J. Thoracic Surg.*, 1940, **10**, 1–7.
3. Skinner, G. F., and Hobbs, M. E. Intrathoracic cystic lymphangioma. *J. Thoracic Surg.*, 1936, **6**, 98–107.
4. Johnston, L. M. Congenital ciliated columnar epithelial cysts of the mediastinum. *Am. J. Dis. Child.*, 1938, **56**, 313–320.
5. Ewing, J. Neoplastic Diseases. W. B. Saunders Co., Philadelphia & London, 1940, ed. 4, p. 1062 *et seq.*
6. Ingraham, F. D., and Scott, H. W., Jr. Spina bifida and cranium bifidum. V. The Arnold-Chiari malformation. *New England J. Med.*, 1943, **229**, 108–114.
7. Hennig, C. Cystis intestinalis, Cystis citra oesophagum bei einem Neugeborenen. *Zentralbl. f. Gynäk.*, 1880, **4**, 398–399.
8. Roth, M. Ueber Missbildungen im Bereich des Ductus omphalomesentericus. *Virchows Arch. f. path. Anat.*, 1881, **86**, 371–390.
9. Staehelin-Burckhardt, A. Über eine mit Magenschleimhaut versehene Cyste des Ösophagus. *Arch. f. Verdauungskr.*, 1909, **15**, 584–603.
10. Schmincke, A. Intramesenteriale und intrathorazische Enterokystombildung, kombiniert mit abnormer Lungenlappung und durch Keilwirbel bedingter kongenitaler Skoliose der oberen Hals- und Brustwirbelsäule. *Virchows Arch. f. path. Anat.*, 1920, **227** (suppl.), 12–27.
11. Mixter, C. G., and Clifford, S. H. Congenital mediastinal cysts of gastrogenic and bronchogenic origin. *Ann. Surg.*, 1929, **90**, 714–729.
12. Smith, R. E. A case of mediastinal dermoid cyst in an infant. *Guy's Hosp. Rep.*, 1930, **80**, 466–469.
13. Fischer, W. Über eine Cyste in der rechten Pleurahöhle. *Virchows Arch. f. path. Anat.*, 1930, **275**, 711–716.
14. Entz, B., and Orosz, D. Über die intrathorakalen Zystenbildungen. *Frankfurt. Ztschr. f. Path.*, 1930, **40**, 229–246.
15. Poncher, H. G., and Milles, G. Cysts and diverticula of intestinal origin. *Am. J. Dis. Child.*, 1933, **45**, 1064–1078.
16. Stoeckel, K. H. Über einen Fall von intrathorakaler Entodermcyste im Mediastinum posterius bei einem Neugeborenen. *Zentralbl. f. Gynäk.*, 1935, **59**, 2178–2183.
17. Black, R. A., and Benjamin, E. L. Enterogenous abnormalities; cysts and diverticula. *Am. J. Dis. Child.*, 1936, **51**, 1126–1137.

18. Brass, K. Über einen Fall von intrathorakalem Enterocystom zugleich ein Beitrag zur Pathogenese. *Frankfurt. Ztschr. f. Path.*, 1936–37, **50**, 26–33.
19. Böss, C. Kongenitale, mit Magenschleimhaut ausgekleidete Mediastinalcyste mit in die Lunge penetrierendem chronischem Ulcus pepticum. *Virchows Arch. f. path. Anat.*, 1937, **300**, 166–179.
20. Guillery, H. Eine in die Wirbelsäule eingewachsene mediastinale Zyste (Vorderdarmzyste). *Zentralbl. f. allg. Path. u. path. Anat.*, 1937–38, **69**, 49–53.
21. Seydl, G. N. Eine kongenitale Magenwandcyste im Mediastinalraum mit in die Lunge perforiertem Ulcus pepticum. *Frankfurt. Ztschr. f. Path.*, 1938, **52**, 346–354.
22. Nicholls, M. F. Intrathoracic cyst of intestinal structure. *Brit. J. Surg.*, 1940–41, **28**, 137–143.
23. Carlson, H. A. Congenital cysts of the mediastinum. *J. Thoracic Surg.*, 1943, **12**, 376–393.
24. Kerr, H. H., and Warfield, J. O., Jr. Intrathoracic dermoids, with the report of a case of total extirpation at one sitting by a new method of thoracotomy. *Ann. Surg.*, 1928, **88**, 607–629.
25. Evans, A. Developmental enterogenous cysts and diverticula. *Brit. J. Surg.*, 1929–30, **17**, 34–83.
26. Bazin, A. T. Mucous cyst of caecum (congenital). Primary intussusception; general considerations. *Canad. M. A. J.*, 1925, **15**, 130–137.
27. McLanahan, S., and Stone, H. B. Enterogenous cysts: report of two cases associated with the rectum. *Surg., Gynec. & Obst.*, 1934, **58**, 1027–1029.
28. v. Wyss, H. Zur Kenntniss der heterologen Flimmercysten. *Virchows Arch. f. path. Anat.*, 1870, **51**, 143–144.
29. Stilling, H. Eine Flimmercyste des Mediastinum anticum. *Virchows Arch. f. path. Anat.*, 1888, **114**, 557–560.
30. Zahn, F. W. Ueber mit Flimmerepithelien ausgekleidete Cysten des Oesophagus, der Pleura und der Leber. Beitrag zur Lehre von den angebornen Mucoidcysten. *Virchows Arch. f. Path. Anat.*, 1896, **143**, 170–187.
31. Rau, F. Casuistische Mittheilungen von der Prosectur des Katharinenhospitals in Stuttgart. III. Flimmerepithelcyste des Oesophagus. *Virchows Arch. f. path. Anat.*, 1898, **153**, 26–29.
32. Mohr. Zur Kenntnis der Cysten des Mundbodens und des Osophagus. (Cited by Staehelin-Burckhardt.[9])
33. Dürck. Flimmerepithelzyste des Oesophagus. *München. med. Wchnschr.*, 1907, **54**, 2165.
34. Kraus. Erkrankungen des Mundhöhle und der Speiseröhre. (Cited by Staehelin-Burckhardt.[9])
35. Coesfeld, H. Ueber Flimmerepithelcysten des Oesophagus. Inaugural Dissertation. L. Handorff, Kiel, 1891.
36. v. Skopnik, A. Ueber Epithelcysten des Oesophagus. Inaugural Dissertation. C. Wolf & Son, München, 1907.
37. Keibel, F., and Mall, F. P. Manual of Human Embryology. J. B. Lippincott Co., Philadelphia & London, 1912, **2**, 362.
38. Keibel, F., and Mall, F. P. Manual of Human Embryology. J. B. Lippincott Co., Philadelphia & London, 1912, **2**, 312.
39. Fitz, R. H. Persistent omphalo-mesenteric remains; their importance in the causation of intestinal duplication, cyst-formation, and obstruction. *Am. J. M. Sc.*, 1884, **88**, 30–57.
40. Lewis, F. T., and Thyng, F. W. The regular occurrence of intestinal diverticula in embryos of the pig, rabbit, and man. *Am. J. Anat.*, 1907–08, **7**, 505–519.

[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 184

FIG. 1. Photograph showing the size of the cyst and its relationship to the right pleural cavity. The esophagus is held aside.

1

Olken Gastro-enteric Cysts of the Mediastinum

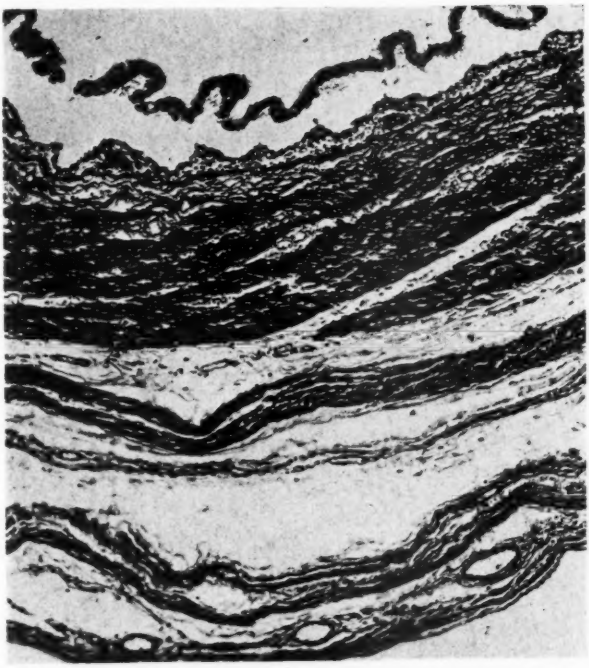
PLATE 185

FIG. 2. A section of the cyst wall showing a simple epithelial lining, one cell in thickness. The mucus-secreting cells are not ciliated. Hematoxylin and eosin stain. × 86.

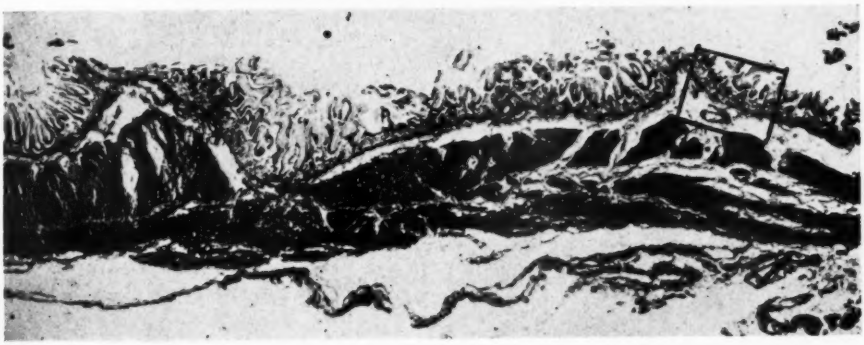
FIG. 3. A second section of the cyst wall showing a more highly developed mucosa. The arrangement of the muscle layers is well shown. Hematoxylin and eosin stain. × 8.

FIG. 4. A detailed view of the mucosa shown in Figure 3. The mucus-secreting epithelium is supported by a loose glandular layer containing acid cells. Hematoxylin and eosin stain. × 160.

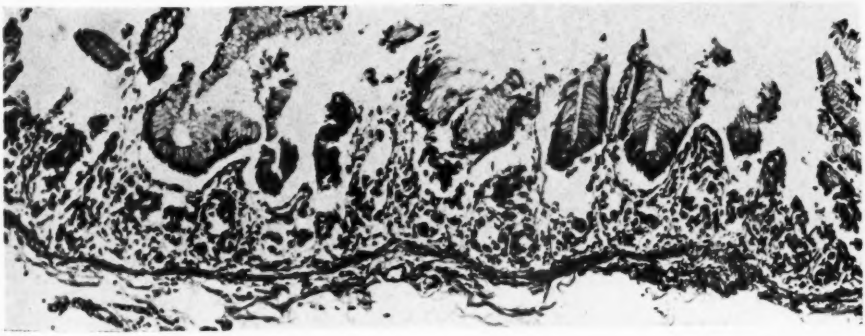
2



3



4



Olken Gastro-enteric Cysts of the Mediastinum

DIFFUSE GLOMERULONEPHRITIS FOLLOWING REVACCINATION FOR SMALLPOX*

PETER A. HERBUT, M.D.

(From the Clinical Laboratories, Jefferson Medical College Hospital, Philadelphia, Pa.)

In the winter of 1942–43, following an outbreak of smallpox in southeastern Pennsylvania, many thousands of people were vaccinated. While most of these suffered no ill effects, there were occasional patients who did develop untoward sequelae. Locally there were at least two cases of generalized vaccinia and one of fatal diffuse glomerulonephritis. The latter is of particular interest because a thorough search of the medical literature, as far back as 1900, discloses no report of such a fatality. There is only one record, that of Saldún [1] in 1932, who described a boy, 8 years old, who developed unequivocal signs and symptoms of acute diffuse glomerulonephritis 14 days after vaccination, and who later made an uneventful recovery. The following is therefore the first case to be reported with autopsy findings.

REPORT OF CASE

A white laborer, 64 years old, was vaccinated on January 11, 1943, together with many other fellow employees. On January 15th he developed a generalized "macular" rash. He was first seen by the referring physician on January 18th because of pain in the lower back. By this time the original eruption had almost completely resolved but there was a marked reaction at the site of vaccination and there were now present scattered pustules in the skin of the same arm. The blood pressure was elevated. Urinalysis was first performed on February 4th, although several days previously the patient had noted that the urine was grossly discolored. It showed a specific gravity of 1006, 4 plus albumin, coarsely granular casts, pus cells and erythrocytes. Bed rest did not alleviate the pain in the back, which radiated anteriorly and to the groin. The referring physician stated categorically that prior to the vaccination, or during the present illness, the patient did not have an upper respiratory infection of any kind. He had been vaccinated only once before and that was 30 years previously.

He was admitted to Jefferson Hospital on February 9th, 29 days after vaccination, in a semicomatose state. Physical examination disclosed a dry, red and fissured tongue; a "uremic" odor to the breath; a distended and tympanitic abdomen and slight pretibial pitting edema. The throat was normal. The bladder was catheterized but yielded only ½ oz. of turbid urine which on examination showed the same constituents as on February 4th. On February 13th the posterior and lateral walls of the pharynx became edematous and the right tonsil was somewhat congested. While the pharyngeal edema gradually subsided until on February 16th it was almost completely resolved, the pretibial edema became increasingly severe. By February 17th the patient was totally irrational and stuporous and he died 37 days after the vaccination.

During his brief stay in the hospital the temperature ranged from 99° to 101° F.; the pulse from 80 to 110 per minute; the respirations from 20 to 36 per minute; and the blood pressure from 160/80 to 200/90 mm. of Hg. The urine repeatedly showed albumin, casts, pus cells and erythrocytes. The urinary output gradually

* Received for publication, January 24, 1944.

decreased from 1580 to 200 cc. per 24 hours while the corresponding intake of fluids varied from 4000 to 1520 cc. per 24 hours. The blood showed anemia and a leukocytosis of 20,000. The nonprotein nitrogen rose from 55 mg. per 100 cc. of blood on admission to 117 mg. per 100 cc. 2 days before death. Two days after admission the total blood proteins were 4.4 gm. per 100 cc., while the albumin was 2.7 gm. per 100 cc. and the globulin was 1.7 gm. per 100 cc. On February 14th a culture from the throat disclosed *Streptococcus viridans, Streptococcus anhaemolyticus* and *Neisseria catarrhalis.*

Gross Examination

Necropsy was performed 3½ hours after death. The skin was normal.

The left kidney weighed 295 gm. and measured 12.5 by 8 by 6 cm.; the right weighed 275 gm. and measured 12 by 7 by 5.5 cm. Both were of normal shape and showed no evidence of previous disease. They were firm. The capsules stripped easily leaving smooth, moist, glistening, reddish brown surfaces, scattered throughout which were numerous bright red petechiae. Cut surfaces showed sharp edges and broad cortices and medullas (Fig. 1). They were also reddish brown and disclosed numerous petechiae. The corticomedullary demarcations were obscured. The vessels were prominent but not sclerotic. The peripelvic fat tissue was not increased. There was no dilatation of the calices, pelves, or ureters, but all of the mucosal surfaces to within 6 cm. of the entrance of the ureters into the bladder were intensely hemorrhagic. The mucosa of the left ureter, particularly in the lower part of the hemorrhagic portion, showed in addition many small vesicles which measured as much as 2 mm. in diameter. The walls of the calices, pelves and ureters were thick and indurated.

The urinary bladder was large but empty and collapsed. Its mucosa was intensely hemorrhagic throughout except for the superficial portions of the rugae which were black. The entire urethra, the prostate, seminal vesicles, epididymides and testes were normal.

The heart weighed 590 gm. It showed considerable hypertrophy of the left ventricle and grossly smooth pericardial surfaces. The posterior and inferior pharynx, the larynx, trachea and bronchi showed no inflammation. There was pulmonary congestion and edema but no pneumonia. The spleen weighed 320 gm. The rest of the thoracic and abdominal organs and the spinal cord were grossly normal. Permission to examine the head was not granted.

Microscopic Examination

Microscopic sections of the kidneys showed an involvement of every glomerulus by an inflammatory or proliferative process (Figs. 2 and 3). While some of the glomeruli were diffusely infiltrated with polymor-

phonuclear leukocytes, others showed only focal collections of such cells. Every glomerulus showed an increase of both endothelial and epithelial cells. There were many adhesions between the tufts and Bowman's capsules. The epithelium of the latter showed beginning or advanced proliferation frequently forming definite and conspicuous glomerular crescents (Fig. 3). Scattered throughout many of the tufts and crescents there were considerable numbers of erythrocytes. Occasionally there were also small, irregular collections of pink-staining amorphous or fibrinoid material intermixed with nuclear fragments and polymorphonuclear leukocytes. While in most glomeruli Bowman's space was completely obliterated by either the tuft or crescent, or both, occasionally it was still apparent and contained erythrocytes and polymorphonuclear leukocytes.

Many of the tubules were slightly or markedly dilated and showed varying degrees of tortuosity. The lining epithelial cells were usually intact. Some were greatly attenuated, flat and contained within their cytoplasm granular brown material. Others were slightly or greatly swollen and were filled with granular or globular, deep-pink-staining, sharply circumscribed bodies which with Nile blue sulfate stained deep blue to purple. There were no vacuoles. Almost all of the tubules, both convoluted and collecting, were filled with casts composed most often of erythrocytes or polymorphonuclear leukocytes and less frequently of hyaline or amorphous material, hemoglobin or epithelial cells. The interstitial tissue showed some diffuse edema and marked congestion of the capillaries. There was a homogeneous sprinkling with polymorphonuclear leukocytes and lymphocytes, and less frequently condensations of these cells into small foci. None of the arteries or arterioles showed any sclerosis or obliterating endarteritis. Very occasionally a small or large arteriole disclosed an acute necrotizing arteriolitis which was strikingly similar to the lesion of periarteritis nodosa (Fig. 4). There were fibrinoid necrosis of the media, some proliferation of the intima but more of the adventitia, and a diffuse infiltration with polymorphonuclear leukocytes and occasional eosinophils and eosinophilic plasma cells. Some vessels in addition contained thrombi in their lumina. In a few arterioles the intima and media were normal but the adventitia and immediately surrounding connective tissue were diffusely infiltrated with cells of inflammatory origin. None of the vessels showed any evidence of healing. Many sections of each kidney stained with Giemsa's stain, and eosin and methylene blue, failed to show inclusion bodies or bacteria of any kind.

Histologic sections of the pelves and ureters were essentially similar. Although the mucosa was mostly denuded, the remaining nests of epi-

thelial cells were normal. There was marked edema of the entire wall, congestion of the capillaries and in the submucosa hemorrhagic extravasation in large areas. There was in addition a diffuse but light infiltration of all of the coats with polymorphonuclear leukocytes and a very occasional eosinophil or eosinophilic plasma cell. Many of the arterioles throughout the entire wall, but particularly the larger ones in the serosa, showed advanced necrotizing arteriolitis of the same nature as that found in the kidneys and previously described. Here, even more than in the kidneys, the degree of vascular involvement was out of all proportion to the amount of inflammation in the adjoining tissue. Sections of the urinary bladder were fundamentally the same as those of the ureters and pelves. There was, however, less sloughing of the epithelium, more extensive hemorrhage and much less inflammation. The arterioles in the outer portion of the wall showed necrotizing arteriolitis similar to, but less extensive than, that found in the kidneys, pelves and ureters. Sections of the lower urinary tract stained with Giemsa's stain, and eosin and methylene blue, as in the kidneys, failed to disclose inclusion bodies in the remaining epithelial cells or bacteria in any of the tissues.

Microscopic sections of the heart showed a uremic pericarditis composed of fibrin, mononuclear cells, a few polymorphonuclear leukocytes and proliferated mesothelial cells. There were no bacteria. The liver revealed focal areas of degeneration, some fatty metamorphosis and a moderate increase of cellularity about the portal radicles. Necrotizing arteriolitis was not found in any of the organs outside of the urinary tract.

Discussion

Because the patient had been in perfect health before the vaccination, there is little doubt that the nephritis was directly related to this procedure. Only 37 days elapsed from the time of vaccination to the time of death, but the changes in the kidneys are entirely compatible with an illness of 5 weeks' duration. Experimentally, it has been shown repeatedly that rabbits develop clinical manifestations of nephritis 5 to 8 days after injection of nephrotoxic duck serum.[2] In the case reported here, back pain developed within 1 week after vaccination and, although the urine was not examined until 2½ weeks later, the nephritis was undoubtedly ushered in with the pain in the loin. Experimentally, it has been shown also that proliferative changes in the glomerular tufts and capsules are clearly evident by the sixth to eighth day after injection of nephrotoxic serum and that by the tenth day definite glomerular crescents are present. Thus pathologically, too, the

renal lesions as described are of such a nature that the available time is well beyond the minimal limit necessary for such changes to develop.

Today, thanks to vaccination, one does not see large epidemics of smallpox such as were prevalent at the turn of the century and before. The adequate post-mortem accounts of the fatalities in those epidemics, however, disclose a striking similarity between some cases of variola and the case of post-vaccination nephritis reported here. All authors agree that ordinarily variola produces no characteristic lesion in the kidneys. Lillie,[3] in summarizing the renal changes which have been described, listed focal necrosis, interstitial nephritis of the type seen in scarlet fever, proliferation of the glomerular epithelium with degeneration of the tubules, degeneration of the tubules alone, acute suppurative nephritis and acute glomerulonephritis. He also stated that most observers have been unable to produce any changes in the kidneys of experimental animals. Councilman, Magrath and Brinckerhoff,[4] in 1904, described 5 cases of acute glomerulonephritis encountered in 54 necropsies of variola which, as far as can be judged from their description, were very similar to the case described here. In one of these the duration of the disease was only 10 days. They further stated that: ". . . . in both variola pustulosa hemorrhagica and in purpura variolosa there is extensive hemorrhage into the submucosa of the pelvis." In 1903, Perkins and Pay [5] reported on 45 autopsies. Of the 3 subjects that had purpura, 2 showed marked hemorrhage into the submucosa of the pelvis. In 212 autopsies reported by Unruh [6] in 1872, 28 showed hemorrhages into the pelves and, in most of these, also in the upper halves of the ureters. Twelve of the 28 patients had hemorrhagic variola while 16 did not. One case also showed hemorrhage into the urinary bladder. Microscopically the epithelium was intact, or denuded and distorted by the massive submucosal hemorrhage. The bleeding was thought to come from the engorged subepithelial capillary network and vessels of the mucosa. In his account Unruh did not speak of the presence or absence of either inflammation or arteriolitis. Finally Ponfik,[7] in 1872, stated that hemorrhages may be present in the calices or pelves of the kidney, the upper halves of the ureters and the urinary bladder.

It is evident from this summary of the literature that, although consistent pathologic lesions do not occur in the urinary tract in all fatal cases of variola, those kidneys that do show noteworthy changes present a somewhat characteristic pattern. Briefly it may consist of a diffuse glomerulonephritis with massive hemorrhages into the calices and pelves of the kidneys, the upper halves of the ureters and occasion-

ally the bladder mucosa. These are precisely the lesions which occurred in the case of post-vaccinial nephritis reported here, except that in this case there was, in addition, severe necrotizing arteriolitis. Why the hemorrhages should be limited to the upper halves of the ureters is not at all clear. Because, however, the inflammatory process in the walls of the calices, pelves, ureters and bladder was relatively slight, and because necrotizing arteriolitis was, in contrast, very severe, the arterial lesions are considered as the cause of the massive hemorrhages. To draw the above comparison between the changes in the urinary tract of cases of variola and those of cases of vaccinia is not so far fetched as may at first glance appear, for there is at present much evidence which shows the close association of the viruses of variola and vaccinia and, in fact, that ". . . . variola can be transformed into vaccinia." [8]

Although there is no doubt that the diffuse glomerulonephritis in the case at hand was related to the vaccination, the exact mechanism involved is more difficult to ascertain. Today, all available evidence, both clinical and experimental, indicates that diffuse glomerulonephritis in general is an allergic response of the kidneys to some "toxin" and not a result of direct bacterial invasion.[9] It is also well known that substances other than bacteria may be the causative agents. Thus, glomerulonephritis has been repeatedly produced in rabbits by the injection of nephrotoxic duck serum.[2] Rich and Gregory [10] produced typical acute glomerulonephritis in sensitized rabbits by the injection of horse serum. Finally Reimann, Price and I [11] reported two examples of necrotizing arteriolitis and, later, chronic glomerulonephritis in patients with trichinosis, probably as a result of sensitivity to trichina protein.

Because the patient whose case is described in this report was vaccinated 30 years previously, it is assumed that sensitivity developed at that time and that the present vaccination resulted in an allergic reaction which seriously affected the urinary tract. Since each vaccine contained both calf serum and vaccine virus, it is impossible to be absolutely certain which was responsible for the sensitivity and the reaction. The appearance of a macular and then pustular rash, however, strongly suggests generalized vaccinia rather than serum disease. The failure to find inclusion bodies in the kidneys or in the rest of the urinary tract is not surprising for, drawing a parallel with other cases of diffuse glomerulonephritis, such renal lesions should be the result of sensitivity to the vaccine protein and not a direct action of the virus on these tissues. Recently, there have been several reports in the French literature [12-14] of acute nephritis following vaccination for typhoid and paratyphoid A and B. Since recovery ensued in all cases, the type of

renal damage was not definitely established, although the clinical behavior in all cases was that of diffuse glomerulonephritis. This vaccine contained only dead organisms and no serum, and therefore the nephritis was undoubtedly due to sensitivity to bacterial proteins. Indirectly this adds credence to the contention that in the case described here the causative agent was most likely the vaccine virus.

There are at least three reports in the literature which indicate that various allergic reactions may sometimes follow vaccination for smallpox. One is a case of acute diffuse glomerulonephritis reported by Saldún [1] and already referred to. A second is a case of purpura developing 14 days after vaccination in a man, 23 years old, described by Heaton.[15] A third instance is a case reported by McLure [16] in which urticaria followed revaccination of a white girl, 5½ years old. The necrotizing arteriolitis observed in the present case is further evidence of the presence of hypersensitivity, although this, like the nephritis, could be due to either the serum or the vaccinia. In the kidneys such arterial lesions are well known in the late stages of very fulminating acute diffuse glomerulonephritis.[9] They have also been described in other viscera by Helpern and Trubek,[17] Baehr,[18] and others. Experimentally, Rich and Gregory [10] have produced both necrotizing arteriolitis and acute diffuse glomerulonephritis in sensitized rabbits by the injection of horse serum. Arteriolar degeneration and necrosis, but with little or no inflammation, have also been produced by Goldblatt [19] and by Winternitz, Mylon, Waters and Katzenstein [20] by ligating the renal arteries in dogs.

Finally, one often sees cases of chronic diffuse glomerulonephritis as a terminal event without any previous knowledge of the existence of the process and with no known antedating infection.[21] Purely on a speculative basis it seems possible that, because of the widespread practice of vaccination, some persons might develop subclinical diffuse glomerulonephritis which would not be apparent until years later. Lyttle [22] has shown, by using the Addis technic, that the urines of patients with scarlet fever, from 8 to 45 days after the onset, show a quantitative increase of albumin, erythrocytes, pus cells, epithelial cells and casts. In other words, they have subclinical nephritis. By using the same technic it might well be that some patients revaccinated for smallpox would show similar changes in the urine.

Summary and Conclusions

1. A case, with necropsy, of acute to subacute diffuse glomerulonephritis following revaccination for smallpox is described in a white man, 64 years old. The literature contains only one other similar report.

2. Pathologically, in addition to typical renal changes of diffuse glomerulonephritis, there were extensive submucosal hemorrhages in the calyces, pelves, ureters and urinary bladder. The lesions in the urinary tract were very similar to some of those described in large epidemics of variola which occurred at the turn of the century.

3. The causative agent is thought to be a sensitivity to the protein of vaccine virus which was first received 30 years previously, although sensitivity to calf serum cannot be absolutely ruled out.

REFERENCES

1. Saldún, M. L. Glomérulo nefritis difusa en primera fase, post-vacunación antivariólica. *Arch. de pediat. d. Uruguay*, 1932, **3**, 442–447.
2. Kay, C. F. The mechanism by which experimental nephritis is produced in rabbits injected with nephrotoxic duck serum. *J. Exper. Med.*, 1940, **72**, 559–572.
3. Lillie, R. D. Smallpox and vaccinia. The pathologic histology. *Arch. Path.*, 1930, **10**, 241–291.
4. Councilman, W. T., Magrath, G. B., and Brinckerhoff, W. R. The pathological anatomy and histology of variola. *J. M. Research*, 1904, **11**, pp. 35, 105.
5. Perkins, R. G., and Pay, G. O. Studies on the etiology and pathology of variola. *J. M. Research*, 1903, **10**, 163–179.
6. Unruh, C. O. Ueber Blutungen in Nierenbecken und Ureteren bei Pocken. *Arch. d. Heilk.*, 1872, **13**, 289–304.
7. Ponfik, E. Über die anatomischen Veränderungen der inneren Organe bei hämorrhagischer und bei pustulöser Variola. *Berl. klin. Wchnschr.*, 1872, **9**, 508.
8. Van Rooyen, C. E., and Rhodes, A. J. Virus Diseases of Man. Oxford University Press, London, 1940, p. 356.
9. Fishberg, Arthur M. Hypertension and Nephritis. Lea & Febiger, Philadelphia, 1939, ed. 4, pp. 448–453.
10. Rich, A. R., and Gregory, J. E. The experimental demonstration that periarteritis nodosa is a manifestation of hypersensitivity. *Bull. Johns Hopkins Hosp.*, 1943, **72**, 65–88.
11. Reimann, H. A., Price, A. H., and Herbut, P. A. Trichinosis and periarteritis nodosa. Differential diagnosis; possible relationship. *J. A. M. A.*, 1943, **122**, 274–279.
12. Boide, and Simonin. Néphrite à la suite d'une vaccination triple. *Soc. de méd. mil. franç., Bull mens.*, 1937, **31**, 656–658.
13. Melnotte, P., and Duret, M. Néphrite aiguë après vaccination triple associée. *Soc. de méd. mil. franç., Bull. mens.*, 1938, **32**, 105–108.
14. Codvelle, F., Paraire, J., and Jacquier. Réflexions sur les accidents rénaux consécutifs à la vaccination triple. *Soc. de méd. mil. franç., Bull. mens.*, 1938, **32**, 99–105.
15. Heaton, T. G. Purpura associated with vaccination against smallpox. *Canad. M. A. J.*, 1940, **43**, 593–594.
16. McLure, W. B. Urticaria and nervous manifestations following revaccination. *Chinese M. J.*, 1930, **44**, 526–531.

17. Helpern, M., and Trubek, M. Necrotizing arteritis and subacute glomerulonephritis in gonococcic endocarditis. *Arch. Path.*, 1933, **15**, 35–50.

18. Baehr, G. The nature of glomerulonephritis. *Bull. New York Acad. Med.*, 1938, **14**, 53–64.

19. Goldblatt, H. Experimental hypertension induced by renal ischemia. *Harvey Lectures*, 1937–38, **33**, 237–275.

20. Winternitz, M. C., Mylon, E., Waters, L. L., and Katzenstein, R. Studies on the relation of the kidney to cardiovascular disease. *Yale J. Biol. & Med.*, 1939–40, **12**, 623–679.

21. Longcope, W. T. The pathogenesis of glomerular nephritis. *Bull. Johns Hopkins Hosp.*, 1929, **45**, 335–360.

22. Lyttle, J. D. The Addis sediment count in scarlet fever. *J. Clin. Investigation*, 1933, **12**, 95–103.

[*Illustrations follow*]

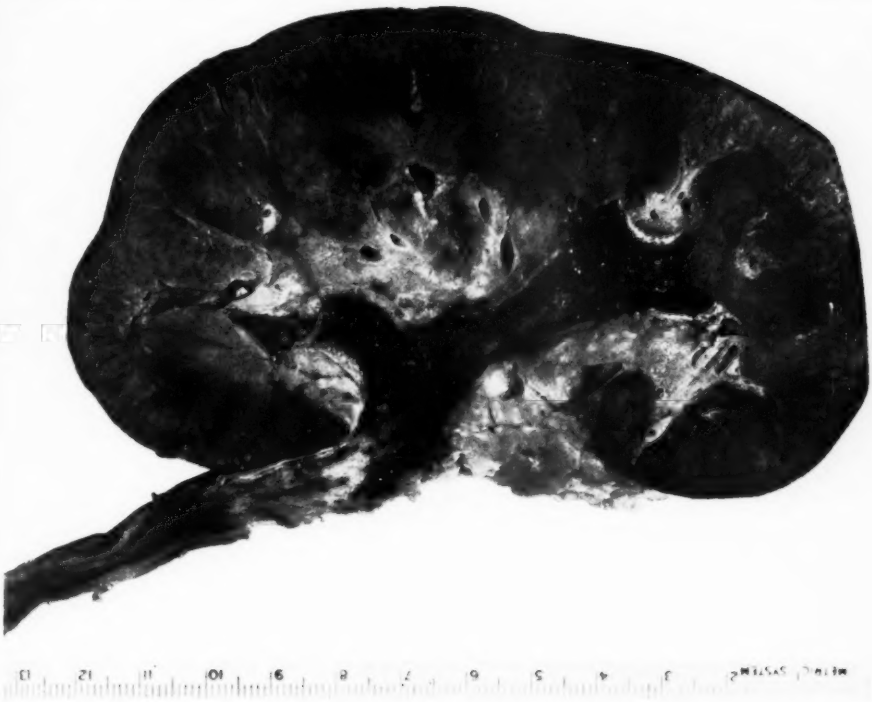
DESCRIPTION OF PLATES

PLATE 186

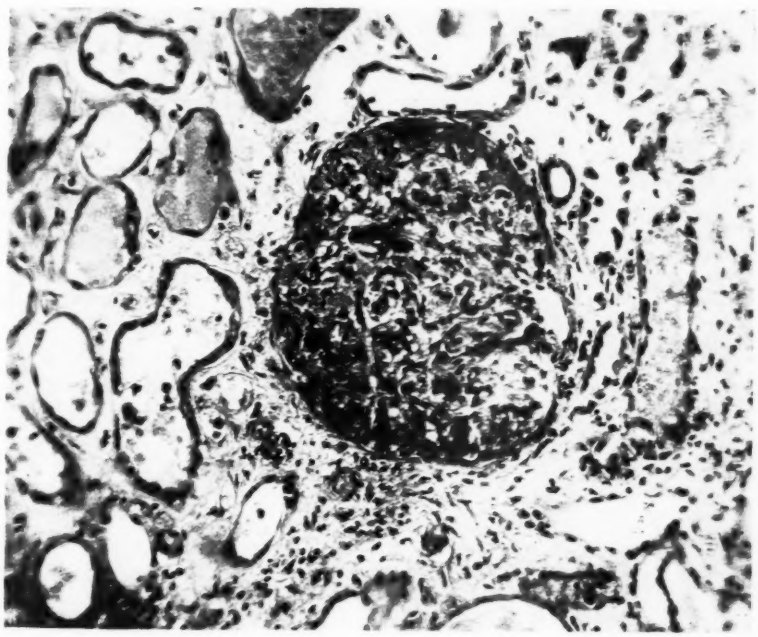
FIG. 1. Cross section of a kidney showing a broad cortex and medulla; numerous petechiae; obscured corticomedullary demarcations and massive submucosal hemorrhages of the calices, pelvis and ureter.

FIG. 2. Photomicrograph of a glomerulus showing a marked proliferation of endothelial and epithelial cells; a diffuse infiltration of the entire tuft with polymorphonuclear leukocytes; marked erythrocytic extravasation about the periphery of the glomerulus; edema and polymorphonuclear leukocytic infiltration of the interstitial connective tissue; dilated tubules; degenerated tubular epithelium and casts composed chiefly of erythrocytes and amorphous material. Hematoxylin and eosin stain. × 200.

1



2



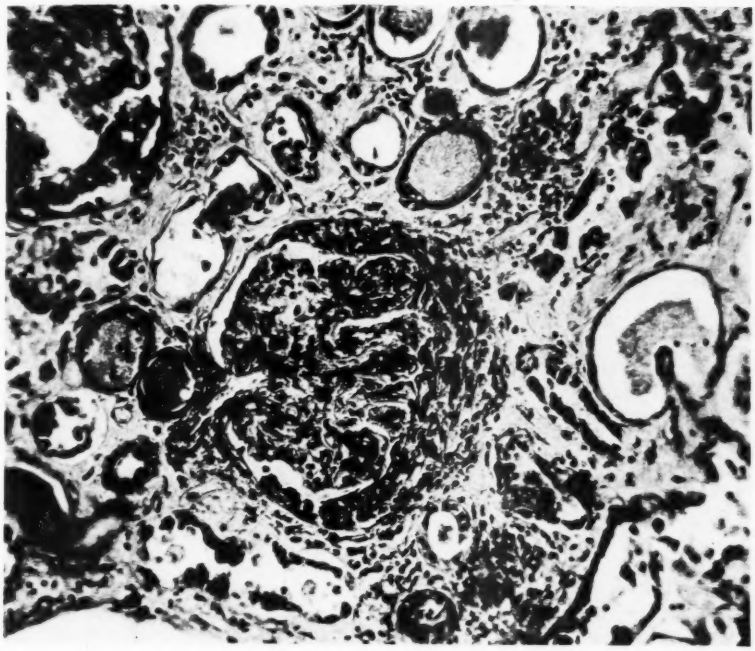
Herbut Glomerulonephritis Following Revaccination

PLATE 187

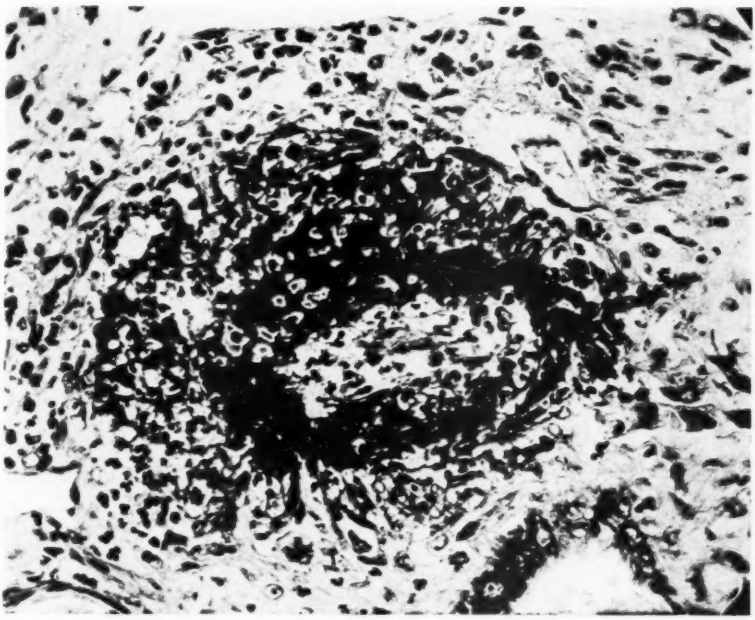
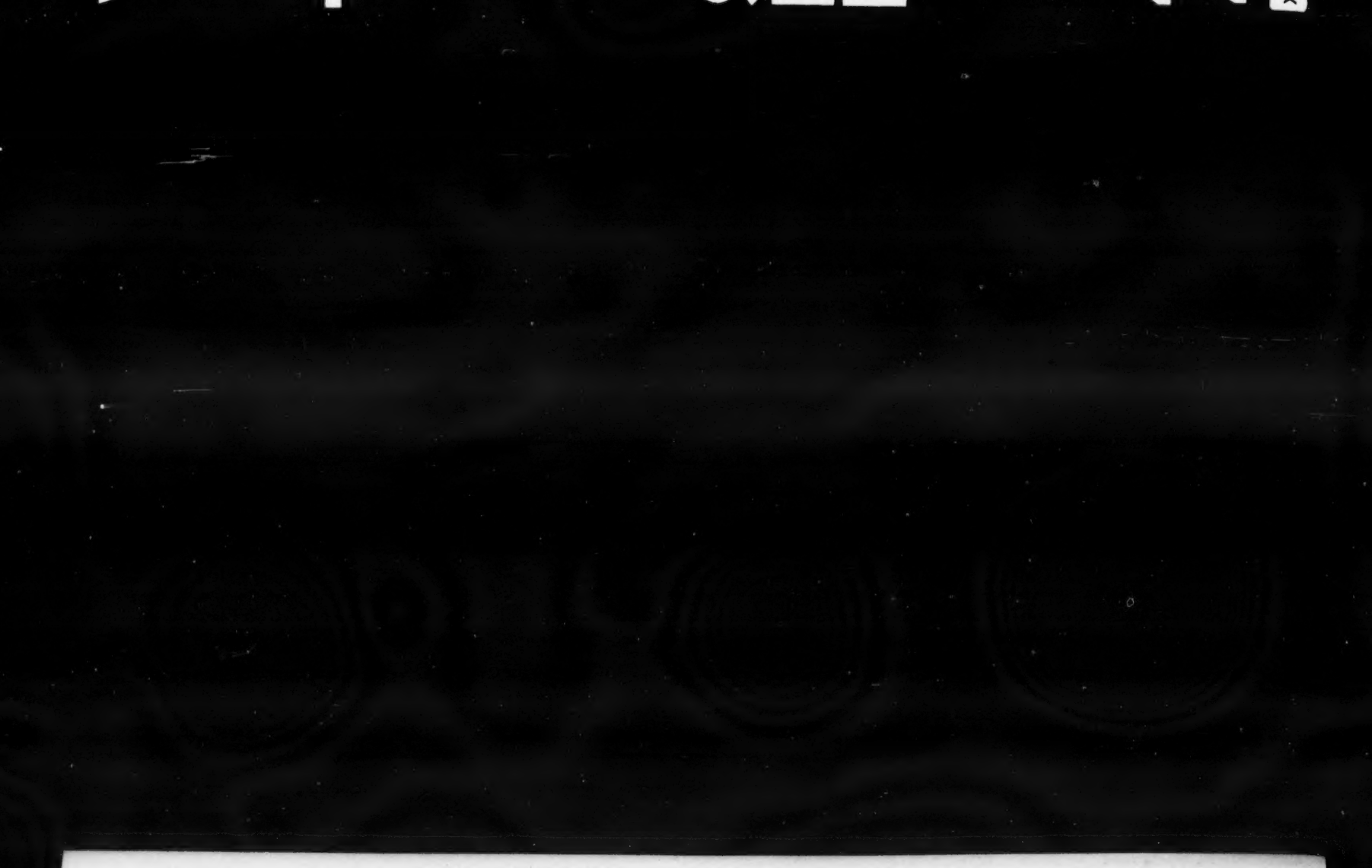
FIG. 3. Photomicrograph of a glomerulus showing essentially the same changes as in Figure 2 and in addition a well formed glomerular crescent. Hematoxylin and eosin stain. × 200.

FIG. 4. Photomicrograph of a small renal arteriole showing intimal and adventitial proliferation; advanced fibrinoid necrosis of the media and a diffuse infiltration with polymorphonuclear leukocytes and scattered eosinophils and eosinophilic plasma cells. There is relatively slight inflammation of the surrounding connective tissue. Giemsa's stain. × 400.

3



4



Herbut Glomerulonephritis Following Revaccination